

# ANNALS OF BOTANY

EDITED BY

DUKINFIELD HENRY SCOTT, M.A., LL.D., D.Sc., F.R.S.

LATELY HONORARY KEEPER OF THE JODRELL LABORATORY, ROYAL BOTANIC GARDENS, KEW

JOHN BRETLAND FARMER, D.Sc., M.A., F.R.S.

PROFESSOR OF BOTANY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

FRANCIS WALL OLIVER, M.A., D.Sc., F.R.S.

QUAIN PROFESSOR OF BOTANY, UNIVERSITY COLLEGE, LONDON

AND

ROLAND THAXTER, M.A., Ph.D.

PROFESSOR OF CRYPTOGAMIC BOTANY IN HARVARD UNIVERSITY, CAMBRIDGE, MASS., U.S.A.

*ASSISTED BY OTHER BOTANISTS*

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# Studies in some East Indian Hepaticae. *Calobryum Blumei*, N. ab E.

BY

D. H. CAMPBELL.

With Plate I and six Figures in the Text.

IN 1830 Nees von Esenbeck described, under the name *Monoclea Blumei*, a remarkable Liverwort collected by Blume in Java. Later the plant was removed from the genus *Monoclea* and named *Calobryum*.

For nearly sixty years no further collections of the plant were made, when it was rediscovered in Java by Goebel.<sup>1</sup> More recently it has been collected by several botanists in the same location, and is also reported from Sumatra.<sup>2</sup>

In 1906 the writer spent several months in Western Java, and near Tjibodas, on the slope of Mount Gedeh, made several collections of this plant. Material from Mount Salak, near Buitenzorg, where Goebel's specimens were obtained, was also examined.

In addition to the Javanese species, there are two others known at present—*C. Mnioides*, (Lindb.) St., comes from Japan, and *C. andinum*, (Spruce) St., occurs in some of the West Indies and in South America. The evidently related genus, *Haplomitrium*, comprising a single species, *H. Hookeri*, is an extremely rare Liverwort found in Great Britain and at a few points on the continent of Europe. These are the only members of the family Calobryaceae, whose relationships with other Hepaticae are very obscure.

Goebel<sup>3</sup> has given an excellent account of the more important vegetative structures of *Calobryum*, but details are lacking of the development of the reproductive organs and embryo; and as the writer's collection afforded very satisfactory material of both male and female plants, as well as a small number of embryos and young sporophytes, it seemed worth while to investigate these somewhat carefully for comparison with the other Hepaticae.

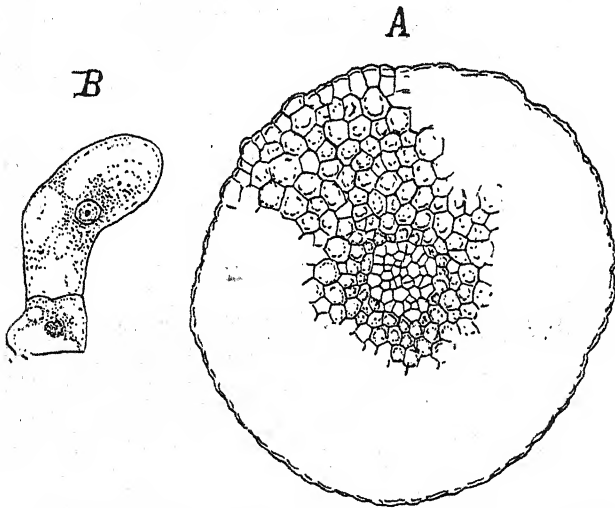
<sup>1</sup> Goebel, K.: Morphologische und biologische Studien. Ann. du Jardin Botanique de Buitenzorg, ix, 1891.

<sup>2</sup> Schiffner, V.: Die Hepaticae der Flora von Buitenzorg, 1900.

<sup>3</sup> Loc. cit.

The results of these investigations show that the reproductive structures differ quite as much from those of the typical Liverworts as do the vegetative characters. Our knowledge of the development of the reproductive organs and sporophyte of *Haplomitrium* are at present too incomplete to make possible a satisfactory comparison with these points in *Calobryum*. Leitgeb,<sup>1</sup> as a result of his studies on *Haplomitrium*, was unable to discover any near relationship with any of the other Hepaticae.

The general aspect of *Calobryum* (Pl. I, Figs. 1-4) is quite different from that of an ordinary Liverwort, since the strongly dorsiventral character, almost universal among Liverworts, is either quite absent, or but slightly developed. The leafy shoot is usually quite upright, and the leaves arranged radially about the axis. There are, however, three rows of leaves



TEXT-FIG. 1. A, Cross-section of a leafy shoot of *Calobryum* ( $\times 56$ ); B, a glandular hair ( $\times 420$ ).

as in the ordinary foliose Liverworts, and very often the leaves of one row are smaller than the others and may be compared with the amphigastria or ventral leaves of the familiar leafy genera. *Calobryum* very much resembles a true moss, such as some of the larger species of *Mnium*, this resemblance being emphasized, especially in the male plants, by the flattened rosette of large leaves surrounding the terminal disc of antheridia.

The leafy shoots arise from extensively branching prostrate stems forming a sort of rhizome. The branches of the rhizome are cylindrical, and quite destitute of rhizoids, but there may be developed short mucilage-secreting hairs, which also occur upon other portions of the plant. Some of the branches of the rhizome begin to develop leaves near the apex, turn upward to the light, and soon assume the character of leafy

<sup>1</sup> Leitgeb, H.: Untersuchungen über die Lebermoose, ii, 1875.

shoots (Fig. 1, k). From the bases of these leafy shoots, as well as from the prostrate portions of the rhizome, horizontal leafless branches are formed, which continue the rhizome, which is thus seen to be sympodial in character.

The stem grows from a tetrahedral apical cell, like that of *Treubia*, or of the foliose Jungermanniales. Goebel figures both longitudinal and transverse sections of the stem apex, and the writer's preparations agree perfectly with Goebel's figures. *Haplomitrium*, to judge from Leitgeb's account and figures, closely resembles *Calobryum* in the form of the apical cell.<sup>1</sup>

Seen in cross-section (Text-fig. 2, F), the apical cell appears triangular in outline, one side being somewhat shorter than the other two. Presumably the shorter side is ventral in position. In longitudinal section the lateral faces are strongly curved, and converge above so that the outer free surface of the apical cell is relatively small (Text-fig. 2, A). Goebel's Fig. 25 of the apex of a rhizome branch shows the apical cell to be deeper and narrower than that of the leafy shoot.

Each segment of the apical cell in the upright shoots gives rise to a leaf. The leaves, as Goebel pointed out, differ from those of most Liverworts in being thickened at the base. In this respect *Calobryum* resembles *Treubia*, but it is not likely that this indicates any near relationship between the two genera.

The first wall in the young segment divides it into an outer and an inner cell (Text-fig. 2, A). From the outer cell is developed the outer thin portion of the leaf, while the inner cell, by further division, gives rise to the thickened leaf-base and the portion of the stem between it and the next older leaf. The line dividing the apical and basal regions of the young leaf is very clearly defined (Text-fig. 2, A-C).

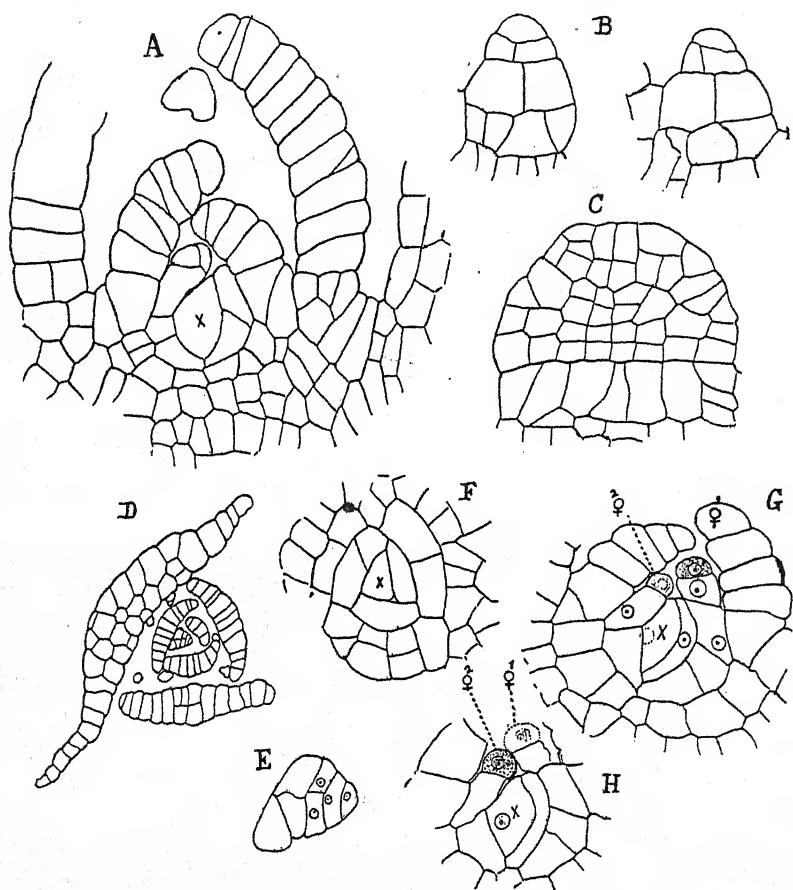
Sections of the very young leaf parallel with the surface (Text-fig. 2, B) show what looks like an apical cell from which a series of basal segments have been cut off. This apical growth, however, is no longer evident in older stages (Fig. C), and the subsequent growth of the leaf is probably due largely to the activity of cells near the base. In the upper part of the leaf the divisions are in two planes only, so that it remains but one cell thick; but in the basal region, where the cells are much larger, divisions may be in all directions, and there is thus formed a thickened basal zone. In the older leaves the thickened basal region merges gradually into the thinner part of the leaf.

The stem, as already stated, consists of two parts, the rhizome-like basal portion and the upright leafy branches. The rhizome, as Goebel pointed out, is sympodial. From the base of the leafy shoots there are produced leafless branches which may themselves give rise to similar ones.

<sup>1</sup> Leitgeb: loc. cit.

Sooner or later the apices of some of these leafless shoots turn upward, develop leaves, and become typical leafy branches upon which are borne the reproductive organs.

Goebel thinks that the leafless branches arise from the apex of the young leafy shoots, but remain dormant until the shoot has elongated,



TEXT-FIG. 2. A, Median longitudinal section of the apex of a leafy shoot of *Calobryum*, showing the apical cell,  $x$  ( $\times 255$ ); B, two sections of a young leaf, cut parallel with the surface ( $\times 255$ ); C, a similar section of an older leaf; D, transverse section, above the apex, showing the leaf arrangement ( $\times 65$ ); E, horizontal section of a young leaf from the same section ( $\times 255$ ); F, apex of the stem in cross-section, showing apical cell,  $x$ ; G, H, two sections of the apex of a branch, showing the first archegonia  $\varphi^1$ ,  $\varphi^2$  ( $\times 255$ ).

so that they seem to be of basal origin. This is contrary to Leitgeb's conclusions for *Haplomitrium*, where he says there is no terminal branching, and that all the branches are intercalary in origin.

The writer has not made a critical study of this point, but from a somewhat careful examination of a number of much-branched rhizomes he

is inclined to believe that Leitgeb's interpretation is the correct one (see Fig. 1).

A notable character of the stem structure of *Calobryum*, pointed out by Goebel, is a differentiation of the tissues into an outer region, or cortex, composed of cells containing numerous starch granules, and a conspicuous central cylinder made up of more or less elongated cells, almost destitute of granular contents (Text-fig. 1, A). A cross-section of the stem resembles closely that of a typical moss, and it is highly probable that the colourless axial cells have to do with water conduction, as Goebel believes to be the case. So far as the writer is aware, no other foliose Liverwort possesses this moss-like stem-structure.

Numerous short mucilage-secreting hairs occur in almost all parts of the plant. They usually consist of a short base, made up of one or two cells, and a terminal secreting cell, which is elongated, pear-shaped, and with dense granular contents (Text-fig. 1, B). Where the stem is perfectly erect, the leaves are inserted horizontally, and one cannot always tell which of the three rows of leaves is the ventral one. In other cases, probably where the shoot is inclined, the leaves are obliquely inserted, and those of one row are decidedly smaller, and may be compared with the amphigastria of the foliose Jungermanniales (Fig. 2). How far this difference in the arrangement of the leaves is due to the influence of light remains to be seen, but it is highly probable, as Goebel suggests, that this is the determining factor.

#### THE REPRODUCTIVE ORGANS.

The reproductive organs are formed in considerable numbers at the end of the leafy shoots, and apparently archegonia and antheridia are never formed together. The leaves surrounding the group of reproductive organs are large, and are especially conspicuous in the male 'inflorescence', which, as indicated before, bears a remarkable resemblance to that of such a moss as *Mnium*.

Within the three large perichaetial leaves surrounding the archegonia there are two or three smaller upright leaves which quite conceal the archegonial group.

The most remarkable feature about the reproductive organs is the extraordinary similarity in the early development of archegonia and antheridium, which for some time are so much alike as to be quite indistinguishable.

Leitgeb<sup>1</sup> states that in *Haplomitrium* the early divisions of the antheridium conform to the usual type of the Jungermanniales; but in *Calobryum* this type is unusual, and in most of the very young antheridia examined the early divisions were exactly like those in the arche-

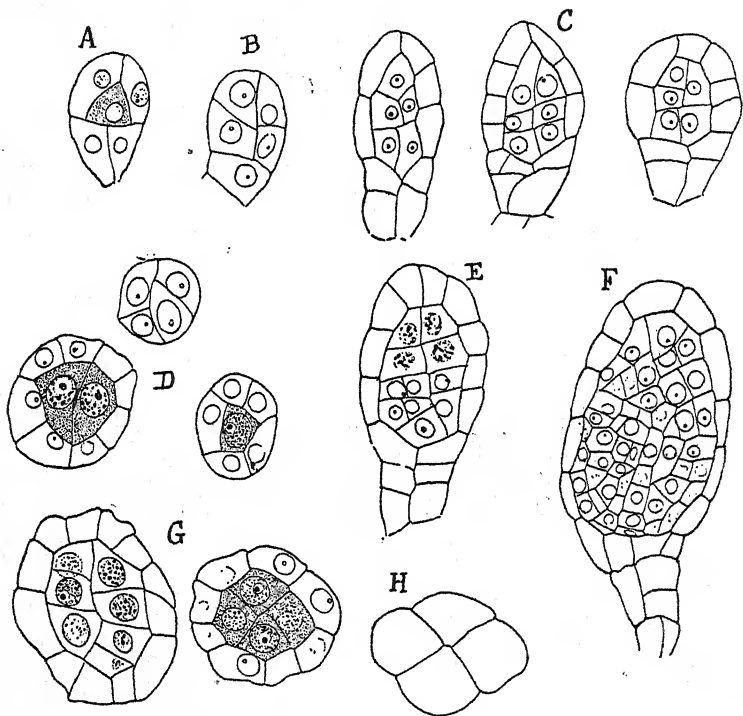
<sup>1</sup> Loc. cit., p. 72.



gonium. Indeed, were it not for the presence of older stages in the same group, one would without hesitation pronounce the youngest stages to be archegonia. In no case, however, were archegonia and antheridia found together in the older receptacles.

Goebel figures a group of old antheridia, but gives no details of their development.

The young antheridium (Fig. 10) consists of a single basal cell and a hemispherical terminal one which gives rise to the body of the antheri-



TEXT-FIG. 3. A, B, Two young antheridia ( $\times 425$ ); B shows a regular octant division; C, three somewhat older antheridia ( $\times 280$ ); D, three young antheridia in cross-section ( $\times 425$ ); E, F, older antheridia ( $\times 280$ ); G, cross-sections of antheridia ( $\times 425$ ); H, cross-section of stalk of mature antheridium ( $\times 280$ ).

dium. The first division wall in the terminal cell is vertical and may be nearly median in position, or it may divide the cell into quite unequal parts (Figs. 11, 12). In the former case the next divisions may follow the usual type of the Jungermanniales, i. e. in each half of the antheridium two intersecting walls are formed, which also intersect the median wall, so that a cross-section of the young antheridium shows two triangular inner cells and four peripheral ones. A cross-section of a somewhat older antheridium of this type is shown in Text-fig. 3, D. This type, however, is much less common than that in which the primary wall is at one side

of the median line, and is intersected by two similar walls, so as to show in cross-section a single triangular cell surrounded by three peripheral ones, exactly as in the young archegonium. These walls may be inclined so as to meet the primary wall, when seen in longitudinal section (Fig. 11), or less frequently they are nearly vertical, and in longitudinal section the axial cell extends to the apex of the antheridium, and later a cap-cell is cut off from it (Figs. 16, 17). The resemblance of these young antheridia to archegonia is quite extraordinary.

A much rarer departure from the type is shown in Text-fig. 3, B. The first divisions were much more like those in the Marchantiales, i. e. there were regular octant divisions before the cutting off of the peripheral cells.

The subsequent development of the antheridium may be briefly summarized. The primary stalk-cell divides by intersecting vertical walls into four, which give rise to the four rows of cells in the stalk of the mature antheridium. The wall of the antheridium consists of a single layer of cells, within which are the numerous sperm cells.

The development of the spermatozoids was followed somewhat in detail, but no notable departure from the type found in other Liverworts was discovered. The nuclei contain eight chromosomes (Fig. 18), and the spermatocytes are not in pairs as is the case in many Hepaticae. The spermatocyte is nearly globular, and surrounded by an evident membrane. The development of the spermatozoid from the contents of the spermatocyte agrees with the accounts given by other investigators for various Hepaticae. As in other cases, the greater part of the body of the spermatozoid is derived from the nucleus, while the cilia arise from the blepharoplast (Fig. 19).

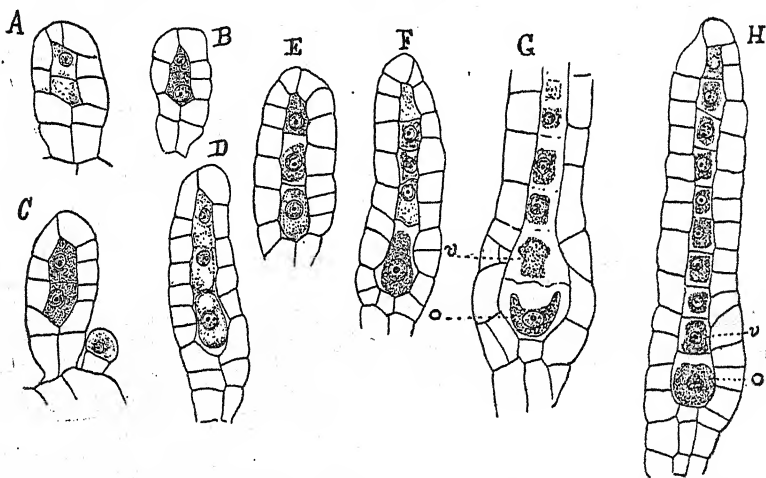
The origin of the first archegonia is exactly the same as in the foliose or 'acrogynous' Jungermanniales (Text-fig. 2, G). Each segment of the apical cell produces a single archegonium, until about half a dozen are produced, when the apical cell is itself transformed into an archegonium, and thus the further growth of the shoot is stopped (Fig. 20). *Calobryum* is thus truly 'acrogynous', while in the related *Haplomitrium* the apical cell does not give rise to an archegonium. Many more archegonia are developed, however, but these are all intercalary, and there is no evident relation between them and the earlier formed ones. The number of archegonia may be considerable, but no accurate count was made. Goebel's estimate of 'thirty or more' is probably correct. Archegonia of very different ages occur together, the younger ones arising close to the base of much older ones.

The early stages of the archegonium, like the antheridium, show remarkable variation. As in other Liverworts, there are first formed three intersecting walls enclosing an axial cell (Fig. 24), but very often these

three walls converge above, and completely enclose the central cell, exactly as occurs in many of the young antheridia, from which they are hardly to be distinguished (Figs. 22, 25). In such archegonia no cap-cell is formed. Sometimes, however, the three primary walls are vertical, and a cap-cell is cut off from the axial cell in the usual way (Fig. 23); but sometimes this takes place very late. In one observed (Text-fig. 5, G), four canal cells had been formed from the axial cell without a cap-cell having been cut off.

A cross-section of the neck in nearly all cases shows but four peripheral cells, instead of the five or six found in nearly all *Hepaticae* (Text-fig. 5, B, F 3). This peculiarity is shown by Goebel in his Fig. 17 a, but he makes no comment upon it.

The neck canal-cells in the mature archegonium are probably in most



TEXT-FIG. 4. A-H. Development of the archegonium ( $\times 280$ ). C shows a very young archegonium close to an older one. v, ventral canal-cell; o, the egg.

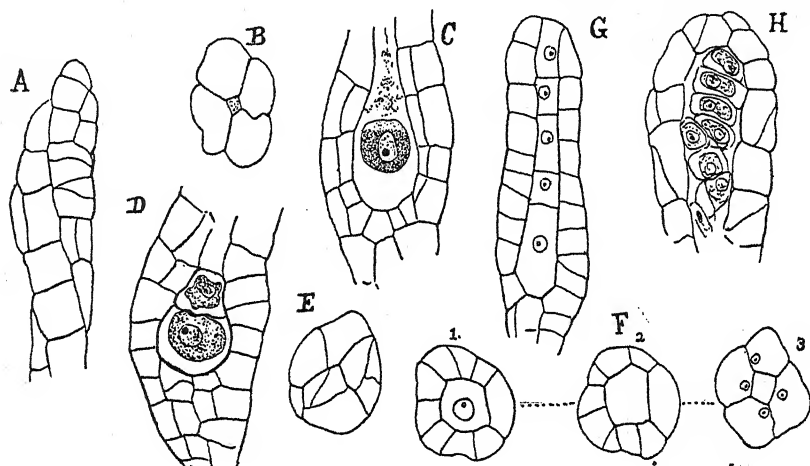
cases sixteen, but as many as twenty may occur. In one case observed (Text-fig. 5, H) there was a marked enlargement of the apical part of the neck, and the canal-cells in this region had divided so as to suggest the sperm-cells of a young antheridium.

When a cap-cell is present it undergoes the usual quadrant division, and there may be a limited number of secondary divisions in the quadrant cells. In the greater number of cases, however, no proper cap-cell is formed, and the terminal cells of the neck are derived from the original peripheral cells and are not the product of a cap-cell cut off from the axial cell, as is the case in all other *Liverworts* that have been described. The neck usually shows a more or less marked torsion (Text-fig. 5, A).

The wall of the venter becomes two-layered at maturity. Its central cell becomes elongated, and the tapering upper portion is cut off by

a definite wall from the lower part, forming the ventral canal-cell. In several cases noted, both the ventral canal-cell and the cell enclosing the egg showed a very conspicuous thickened membrane (Text-fig. 5, D), which would seem to preclude any possibility of fertilization. In other cases the egg degenerated, and there was some evidence that the ventral canal-cell might be fertilized instead of the egg.

The great similarity in the development of the archegonium and antheridium is of interest in connexion with the question of the homologies



TEXT-FIG. 5. A, Surface view of the neck of an older archegonium, showing the torsion of the neck-cells ( $\times 280$ ); B, cross-section of the archegonium neck, showing four peripheral cells ( $\times 280$ ); C, venter of a ripe archegonium; D, venter of an archegonium in which the ventral canal-cell and the egg-cell are enclosed in a thick membrane; E, apex of the neck, seen from the surface; F, three cross-sections of a young archegonium—1, 2, near the base; 3, the neck; G, an archegonium, with four neck canal-cells, in which no cap-cell had been formed; H, apex of an abnormal archegonium, suggesting an antheridium.

of these organs. Goebel<sup>1</sup> has argued that the two are homologous, and this view would certainly be strengthened by the marked resemblances between the archegonium and antheridium in *Calobryum*.

#### THE EMBRYO.

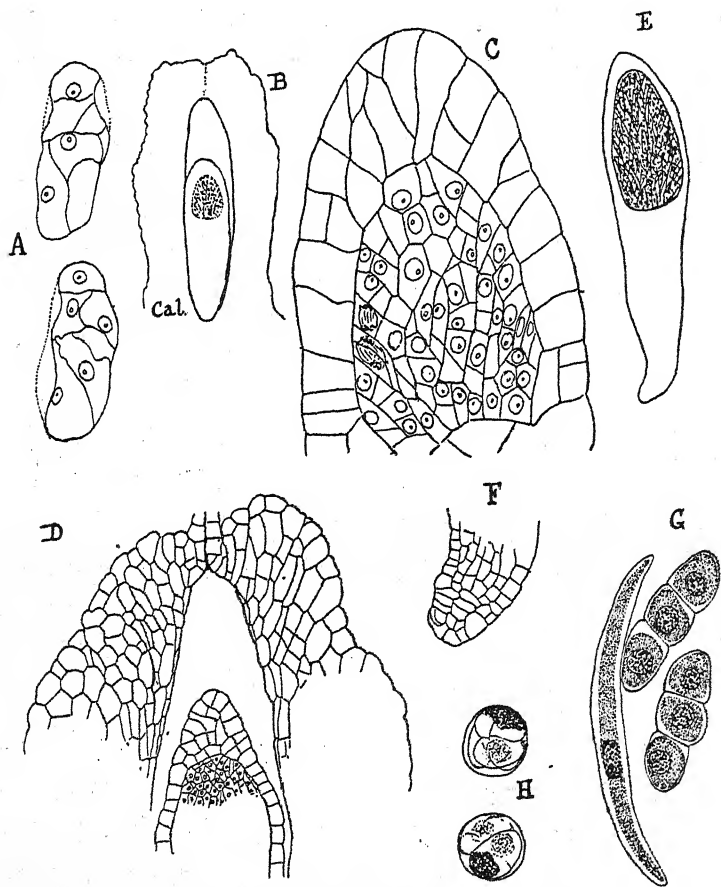
The material collected by the writer showed only a very few young embryos, so that it was not possible to determine with certainty the succession of divisions in the earlier stages.

The youngest specimen found is shown in Text-fig. 6, A. It corresponds pretty closely with the usual type of the Jungermanniales, i. e. there are apparently two transverse divisions before any longitudinal walls are formed. The basal cell, as is usual in the Jungermanniales, probably takes

<sup>1</sup> Goebel, K.: Über Homologien in der Entwicklung männlicher und weiblicher Geschlechtsorgane. Flora, xc. 279-305, 1902.

no part in the future growth of the embryo. The divisions in the upper segments were decidedly irregular, and it was impossible to judge of the relation of these early divisions to the structures of the older sporophyte.

No stages were found between this very early one and those in which the different regions of the sporophyte were clearly differentiated. The



TEXT-FIG. 6. A, Two sections of a young embryo ( $\times 280$ ); B, an older embryo, enclosed in the calyptra, *cal.* ( $\times 38$ ); C, apex of the embryo, shown in B ( $\times 280$ ); D, upper part of calyptra, and young sporophyte ( $\times 66$ ); E, longitudinal section of young sporophyte ( $\times$  about 30); F, the foot ( $\times 66$ ); G, young spore mother-cells and elater ( $\times 425$ ); H, two young spore-tetrads; only one nucleus in each tetrad is normal.

young sporophyte (Text-fig. 6, E) is elongated, the basal portion consisting of a pointed foot which merges insensibly into the seta. The capsule is at this stage slightly enlarged and somewhat pointed. The sporogenous tissue is clearly defined, and is bounded by a single layer of wall-cells, in which respect *Calobryum* resembles the Marchantiales or Sphaerocarpaceae, rather than the Jungermanniales, where the capsule wall is always composed of

more than one layer of cells. Of the Marchantiales, probably *Monoclea* most nearly resembles *Calobryum* in the elongated form of the capsule as well as the long seta.<sup>1</sup>

As the development proceeds, there is the usual differentiation of the archesporial tissue into the spore mother-cells and elaters; but no definite relation of the two to each other could be detected, nor was any structure recognizable as an elaterophore. The spore mother-cells are often in rows of two to four, lying between the elongated young elaters. Both spore mother-cells and elaters show a definite but very delicate membrane, within which lies the contracted protoplast (Text-fig. 6, G).

The apical portion of the capsule wall is thicker, and finally forms a conspicuous prominence or beak, much like that of *Pallavicinia* or *Podomitrium* (Text-fig. 6, D).

No specimens could be found showing the final nuclear divisions of the spore mother-cells, and in nearly all cases the spores were already free. In the only case where the spore tetrads were seen (Text-fig. 6, H) there had apparently been two successive divisions resulting in the bilateral type of spore; but of the four young spores only one contained a normal nucleus, the nuclei of the other three having disintegrated. Whether this appearance is normal is a question; but it may be that only one spore of the tetrad reaches maturity. The ripe spore (Fig. 8) appears quite globular, as might be expected in case only one spore of a tetrad developed. Further study of spore development will be necessary to decide this question.

The ripe spore has a moderately thick membrane, and the surface is marked by numerous short, blunt prominences. The long, slender elaters have a double spiral (Fig. 7).

A very massive calyptra is developed, which finally may reach a length of 15 mm., while the seta is two or three times as long, and bears at its tip the slender pointed capsule, about a centimetre in length, and opening by a slit along one side (Figs. 4, 5).

#### CONCLUSION.

It is evident that *Calobryum* and *Haplomitrium*, although differing in certain particulars, e.g. the position of the archegonia, are closely related, and the establishment of a special family, Calobryaceae, to include these, is entirely warranted. The relationships of the Calobryaceae with the other Hepaticae are very obscure. Goebel regards them as members of a series developed quite independently of the other foliose Hepaticae.

The development of leaves has evidently occurred in several quite independent series among the Liverworts, and the Calobryaceae probably represent the end of such a series, and are not closely related to the foliose

<sup>1</sup> Johnson, D. S.: The Development and Relationship of *Monoclea*. Bot. Gazette, xxxviii. 185-205, 1904.

Jungermanniales. Whether they are most nearly related to the anacrogynous Jungermanniales, or have been derived from forms more like the Sphaerocarpales, is a question. The character of the sporophyte, with its single layer of wall-cells, would suggest the latter hypothesis.

The establishment of a special family, Calobryaceae, is entirely justified, and perhaps an order, Calobryales, should be established, co-ordinate with the Sphaerocarpales, Marchantiales, and Jungermanniales.

The distribution of the species of *Calobryum* indicates that the genus was once more generally distributed than at the present time.

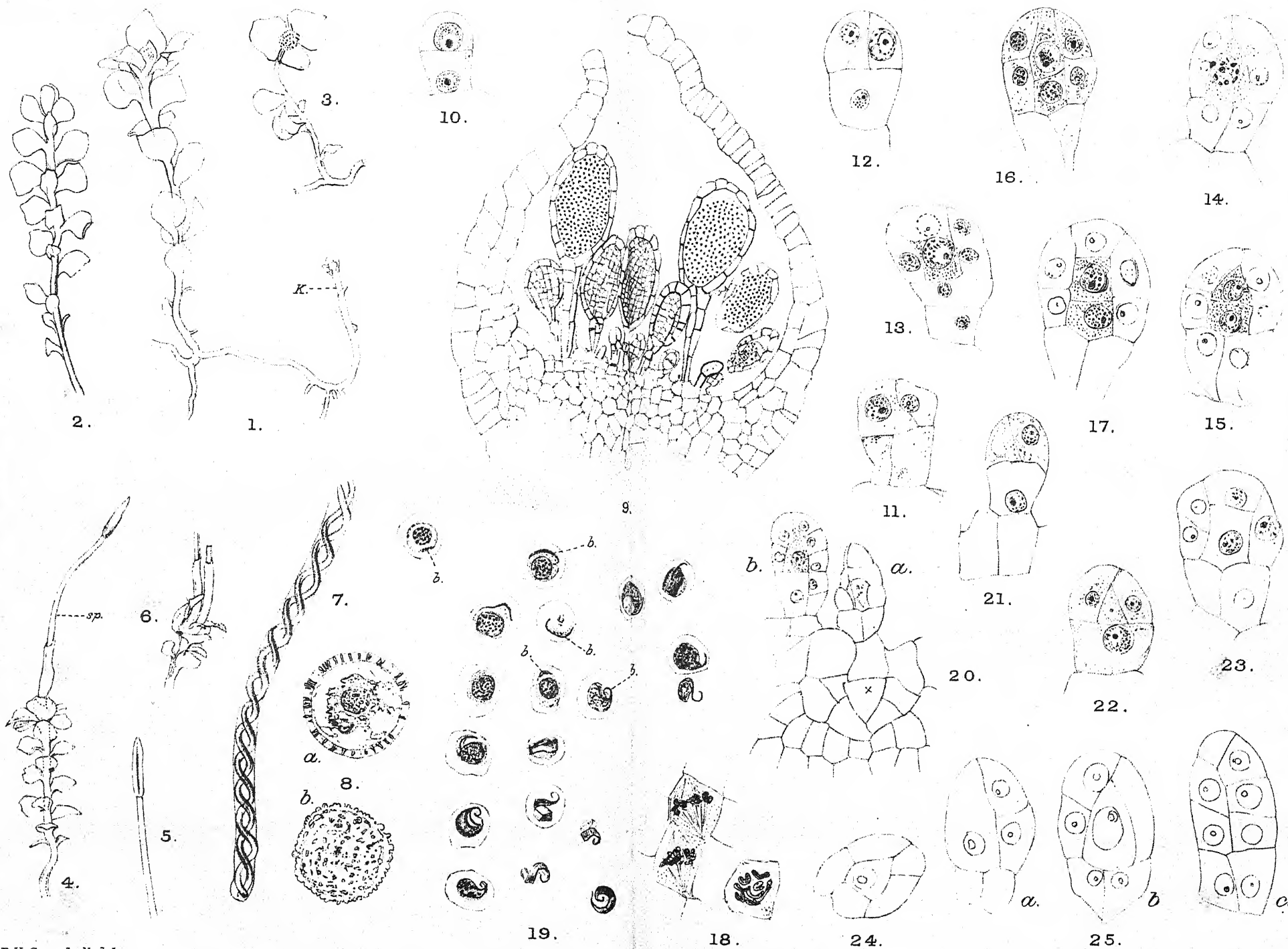
### EXPLANATION OF PLATE I.

Illustrating Prof. Campbell's paper on Studies in some East Indian Hepaticae.

- Fig. 1. Female plant of *Calobryum*, showing a young leafy shoot, *k*, arising from the rhizome.  $\times 2$ .
- Fig. 2. A leafy shoot showing marked dorsiventral habit.  $\times 2$ .
- Fig. 3. A male plant.  $\times 2$ .
- Fig. 4. A shoot with mature sporophyte, *sp*. Natural size.
- Fig. 5. An open capsule.  $\times 2$ .
- Fig. 6. A branch upon which two sporophytes have developed; the upper part, with the capsule, has been broken off.
- Fig. 7. Part of an elater.  $\times 880$ .
- Fig. 8. Two spores.  $\times 880$ . *a*, in optical section; *b*, surface view.
- Fig. 9. Apex of a male plant, with group of antheridia.  $\times 100$ .
- Figs. 10-17. Young antheridia seen in median longitudinal section.  $\times 640$ .
- Fig. 18. Mitosis in young spermatogenic cells, showing eight chromosomes.
- Fig. 19. Development of the spermatozoid. *b*, blepharoplast.
- Fig. 20. Median section of the apex of a branch in which the apical cell has given rise to an archegonium, *a*; the cell *x* is the base of the apical cell; *b*, median section of the archegonium.
- Figs. 21-23. Young archegonia seen in median longitudinal section.  $\times 640$ .
- Fig. 24. Cross-section of young archegonium.
- Fig. 25. Three longitudinal sections of a young archegonium.  $\times 640$ . *b* is the median section.







D.H. Campbell del.

CAMPBELL-HEPATICA.

Huth London.

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# The Relation of Aeration to the Growth and Activity of Roots and its Influence on the Ecesis of Plants in Swamps.<sup>1</sup>

BY

H. F. BERGMAN.

With three Graphs in the Text.

THE effect of various factors on the form and growth of roots has been repeatedly investigated by experimenters during the past fifty years, although little attention seems to have been paid to the effect of the surrounding medium on their functional activity. This is particularly true with reference to their oxygen supply. Very recently, however, Livingston and Free (14, p. 182) have shown that 'the first effect of oxygen deprivation is an interference with the absorption of water by the roots'. Accordingly, in determining the effect of various factors on the growth and distribution of swamp plants, it was decided to make a special study of aeration in such habitats. In so far as possible, exact data have been secured as to the oxygen and carbon dioxide content of lake and swamp waters under normal conditions. The influence of other factors, however, has not been neglected.

The work was in progress about three years. The field work was done mostly during the summer months in the northern part of Minnesota. The experimental work was carried on during the winters of 1914-15 and 1915-16 in the University greenhouse.

## *Effects of Aeration on Submerged Roots.*

Experiments with a number of plants have been performed in the greenhouse to determine the effect of root submergence, through limitation of the air supply, as a factor in the development and activity of roots. In the course of these experiments the plants were grown in the same kinds of soil and under the same conditions of temperature. The kind of water or amount of air supplied were the only factors varied.

Seedlings of bean (*Phaseolus vulgaris* var.), plants of *Impatiens balsamina*, *Pelargonium* sp., *Cyperus alternifolius*, *Ranunculus abortivus* and

<sup>1</sup> Accepted by the University of Minnesota in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

*R. sceleratus*, potted in soil, were placed in vessels of water so that the surface of the water came above the level of the tops of the pots. Duplicate sets in swamp water were also set up. In two days the plants of *Impatiens* began to wilt. In three days they were badly wilted, and in four days were wilted beyond recovery. On the fourth or fifth day plants of *Pelargonium* and *Phaseolus* began to wilt, and if left in water two or three days longer the leaves turned yellow and dropped. *Cyperus* and *Ranunculus* showed no ill effects, but grew vigorously with their roots submerged.

Later the same experiment was repeated, but as soon as the plants showed signs of wilting air or oxygen was supplied. It was found in all cases that the plants, if not too badly wilted, could be restored to normal condition and could be kept growing indefinitely if aeration were continued. After a week or ten days all plants developed new roots at or near the surface of the water, after which the plants were able to live without having oxygen or air artificially supplied. No difference in the behaviour of plants in tap-water as compared with those in swamp water could be noted. For this reason, and also because of the difficulty of obtaining swamp water during the winter months, the use of swamp water in later experiments was discontinued.

#### *Effects of excluding Air from Roots in Soil.*

Two plants of *Pelargonium*, potted in soil, were set up in the following manner. The leafy stems were passed through the mouth of a wide-mouthed bell-jar so that the stem and leaves came out into the air while the roots in the pot were under the bell-jar. The mouth around the stems and the base of the bell-jar were sealed with wax so as to be air-tight. Carbon dioxide was then passed through to replace the air. This was repeated morning and night to maintain an atmosphere of pure carbon dioxide. A watering device was so arranged that water could be added without admitting air at the same time. A slight wilting of one plant was apparent on the second day following, and on the third day the wilting was very evident. Wilting of the second plant did not begin until the fourth day, but on the fifth day it had become very pronounced. The leaves on both plants after wilting became yellow and soon dropped. The yellowing and dropping of leaves continued until the test was ended, ten days after it was begun. The soil was found to be normally moist at the end of the test.

A plant of *Impatiens balsamina* was arranged as just described for *Pelargonium*, and the air replaced by carbon dioxide. On the second day the plant was slightly wilted, and on the morning of the third day badly wilted. The plant was removed at noon on the third day. On the following day the plant appeared as if badly frosted. This plant never recovered.



*Experiments with Ranunculus.*

The following autumn the experiments were repeated on a more extensive scale. Six plants each of *Ranunculus abortivus* and *R. sceleratus*, potted in soil, were placed in vessels of water so that the roots were completely submerged. Three plants of each were left in ordinary garden soil as controls. After three months the plants were all in good condition. It was found, however, that the plants with submerged roots had produced more and larger leaves and considerably more extensive root systems than those in moist soil. The roots in all cases, whether submerged or not, were distributed throughout the soil.

*Experiments with Corn (Zea) and Beans.*

Eighteen pots each of corn and beans were planted in sets of three under the following conditions: in garden soil as a check; in soil, roots submerged; in peat; in peat, roots submerged; in *Sphagnum*; and in *Sphagnum*, roots submerged. After the plants were up the cotyledons of the beans were removed. They were then allowed to grow two or three days before the test was begun. Swamp water was used in watering except for plants in soil, for which tap-water was used. The plants were allowed to grow three months. At the end of this time the plants of both corn and beans growing in soil had made the best development of leaves and roots. The roots extended throughout the soil, tending to mat at the bottom, and were well provided with root-hairs. Bean plants in soil, with roots submerged, developed as many leaves as those in soil not submerged. The leaves soon dropped, however, so that at the end of the test the plants with submerged roots had only one or two leaves each. Corn in soil, with roots submerged, showed little or no retardation in the extent of shoot development. Root development after submergence was less extensive and entirely superficial in both beans and corn.

Beans grown in moist peat or *Sphagnum* developed nearly as much foliage as those grown in soil. The plants, however, were not quite as tall nor as robust. Root development was not as extensive as in plants grown in soil. The roots extended throughout the peat or *Sphagnum*, tending to mass at the bottom of the pots. Root-hairs were present in abundance. Beans and corn when grown in either peat or *Sphagnum*, with the roots submerged, showed a marked inhibition in growth. The stems were more slender and dwarfed. The leaves were usually less numerous and were reduced in size as compared with plants in soil, peat, or *Sphagnum*, the roots of which had not been submerged. Root development was poor. The roots of corn grew much more extensively than those of beans, which seldom reached out to the edge of the pots. They were always less extensive than those of plants in soil with the roots submerged.

*Experiments with Impatiens balsamina.*

Two plants of *Impatiens*, potted in soil, were placed in vessels of water so that the roots were completely submerged. Several plants of *Philotria* were placed in the water surrounding one of the pots. Aeration in this case was provided by the evolution of oxygen from the photosynthetic activity of *Philotria*. The second plant was in water without *Philotria*. Three days later the first plant was in good condition, while the second was wilted. The wilting of the second plant continued for two days more, when it was in such bad condition that it was removed. The first plant was still in good condition. This plant was left two months, during which time it lost about four or five leaves. Ten days after submergence of the roots new roots were coming out along the stem at the surface of the water. These continued to develop, and an examination later showed that the lower roots had died, so that only the superficial ones were alive.

The experiment with *Impatiens* was repeated later, six plants being used. All were potted in garden soil. Four of them were placed in water with the roots fully submerged. Of these four, one was aerated by bubbling air continuously through the water. Another was aerated by putting some *Spirogyra* in the water in which the potted plant was placed. The other two were not aerated. The fifth plant was grown in very wet soil. This was done by placing the pot in a basin of water so that only the bottom of the pot was in direct contact with the water. The sixth plant was grown in moist garden soil as a check. In three days the plants with the roots submerged in unaerated water were badly wilted and were removed. All other plants were in good condition. In five days the plant in water aerated by bubbling air through the water showed a slight yellowing of some of the leaves, but no wilting. The plant aerated by *Spirogyra* was slightly wilted. This was assumed to be due to continued cloudiness, on account of which the liberation of oxygen by *Spirogyra* was reduced to such an extent that the water was insufficiently aerated. On the following day both plants in aerated water were badly wilted, the one in water aerated by *Spirogyra* being in the worse condition. Ten days after the experiment was begun the plants were still living, but each had lost several leaves. The remaining leaves were not wilted. Roots were beginning to develop along the stem at the surface of the water in both plants. The plants grown in soil wet by capillarity, and in moist garden soil, were in good condition.

The experiment was allowed to run three weeks. All the plants except the two in unaerated water survived. Those with roots submerged in aerated water lost several leaves in the first week or ten days. After developing roots at the surface of the water, however, they began to grow again, and apparently normally. Roots below the surface died. Root-hairs were

developed on some of the roots in aerated water. In the plant in soil wet by capillarity roots also developed at the surface. In this case the upper roots made the greatest growth, but all the lower roots were living. In moist garden soil root development was extensive, reaching to all parts of the pot with an abundant development of root-hairs.

*Experiments with Pelargonium and Coleus.*

A similar experiment was carried out with *Pelargonium*. On plants with the roots submerged and not aerated, the leaves began to turn yellow in ten days or two weeks. Shortly afterwards the leaves dropped. The behaviour of the roots of *Pelargonium* was similar to that observed in *Impatiens*. In all cases where the roots were submerged, whether or not the water was aerated, new roots developed at the surface, while the submerged roots died. This was also found to be true of the roots of *Pelargonium* in soil wet by capillarity, although with *Impatiens* the lower roots remained alive. After the development of surface roots the plants again began to grow and produce new leaves. Root development in ordinary moist soil was abundant. *Pelargonium* was found to be less responsive than *Impatiens* in that it did not show wilting as clearly or as quickly. Loss of colour was the first sign of distress. Soon after changing colour the leaves usually dropped.

Plants of *Coleus Blumei* potted in soil, with the roots submerged, showed signs of distress in one or two days. The first evidence was the wilting of the leaves. The leaves after remaining in a more or less wilted condition a few days dropped off, until only two or three small leaves near the top of the stem remained. After ten days or two weeks new roots were always found to have developed from the stem at the surface of the water. Plants in moist soil developed normally.

*Experiments with Vicia Faba.*

An experiment with *Vicia Faba* was tried as follows: Seeds were planted in batteries of six pots each containing garden soil, peat, and *Sphagnum* respectively. As soon as the seedlings had broken through the ground the cotyledons were removed from two plants in each set. From each battery of six, two plants with cotyledons and one with cotyledons removed were placed in vessels of water so that the roots were fully submerged. The other three pots of each battery of six were kept moist, but never to saturation. In four or five days all plants, the roots of which had been submerged, began to wilt. This condition continued for a week or more in varying degree according to the temperature of the greenhouse. In all cases, however, the plants lived. The plants were permitted to grow ten weeks; at the end of this time it was noted that the plants in soil with the roots not submerged had grown most, those in *Sphagnum* least, and

those in peat intermediate. In submerged plants less difference was noticeable, although the same order seemed to hold. Loss of leaves did not occur in any of the plants. Removal of the cotyledons caused a general reduction in the amount of growth. This was least noticeable in plants grown in moist garden loam, and quite evident in plants grown in peat or *Sphagnum*. The reduction in growth was most noticeable in plants grown in peat or *Sphagnum* with the roots submerged.

Plants grown in moist soil showed the greatest development of roots. The form of the root system of plants grown in either peat or *Sphagnum* was similar, and in extent of growth nearly equal to those in soil. The roots of all plants under conditions of submergence were similar. The lower roots were dead, and new roots had developed from the stem at the surface of the ground. The depth of penetration was never more than an inch below the water surface. The longer and more branched roots usually came even nearer the surface. No root-hairs were present on submerged roots, although abundantly present on others.

#### Effect of Root Submergence on Transpiration.

The following experiment was performed to ascertain the effect of root submergence on transpiration: Two plants of *Pelargonium*, potted in soil, were placed in aluminium pots, the tops of which were covered with

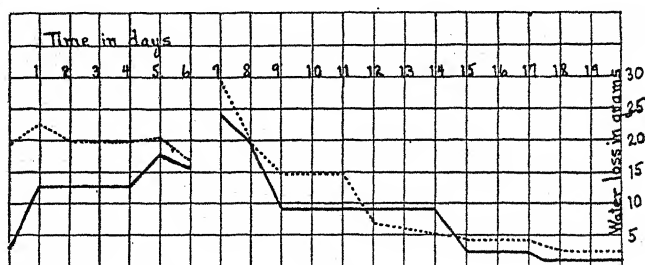


FIG. 1. Graphs showing comparative rates of transpiration of two plants of *Pelargonium*, the broken line for the one in moist soil and the solid line for the one in soil submerged. The break on the sixth day marks the time at which the roots were submerged. On the eleventh day the leaves began to turn yellow, and by the fourteenth day were beginning to drop off. A decrease in the rate of transpiration is evident on both of these days.

sheet rubber to prevent evaporation from the surface of the soil. The plants were weighed each day for a week. The roots were then submerged and the daily weighing continued. The results are shown in the accompanying graph (Fig. 1). On the day following root submergence it was found that the rate of transpiration had greatly increased. The rate, however, fell off rapidly, and after two days had fallen to a point lower than when growing in moist soil. Recording instruments showed that the humidity and temperature conditions in the greenhouse had not changed.



The reduced rate, then, is not to be explained by lower temperature and higher humidity. After two days of the reduced rate of transpiration, the leaves began to turn yellow. From this time the average transpiration rate dropped still lower until at the end of ten days after the roots had been submerged the transpiration rate per day averaged 7.3 grm. for one plant, and 9 grm. for the other. This average rate was maintained three days, at which time the plants began to shed their leaves. In the nine days following, in which weighings were continued, the average daily rate of transpiration was reduced to 2.8 grm. for one plant, and 1.5 grm. for the other. It is to be observed that when the roots of plants had been submerged a decrease in the average daily rate of transpiration soon followed. It is to be noted, further, that this decreased average daily rate of transpiration

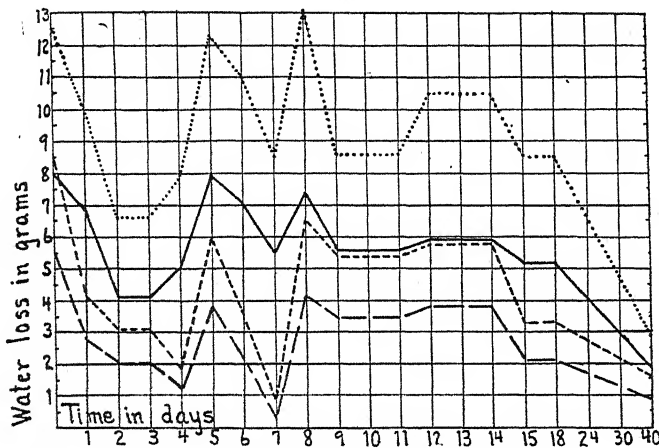


FIG. 2. Comparative transpiration of two seedlings of *Quercus macrocarpa*. The dotted line represents a plant in moist soil, the line of long dashes a plant in submerged soil. Leaf area of former, 100 sq. cm.; of the latter, 64.27 sq. cm. The line of short dashes represents the rate of transpiration of the plant with the smaller leaf area based on 100 sq. cm. leaf surface; the heavy solid line represents the transpiration rate of the plant with 100 sq. cm. leaf area based on the same leaf area as the plant in submerged soil.

continued two or more days *before* the manifestation of any evidence of injury to the plant. These facts indicate clearly that transpiration is greater than absorption. The shedding of leaves is to be regarded as a compensation for the reduced ability to absorb by reduction of the transpiration surface.

Two seedlings of *Quercus macrocarpa* were potted in garden soil in aluminium pots. The roots of one were submerged, while the other was allowed to grow in moist soil. The pots were covered with rubber tissue to prevent surface evaporation, and weighings made. The results are shown in the accompanying graph (Fig. 2).

The plant with the roots in moist soil had a leaf area of 100 sq. cm., and the plant with submerged roots had a leaf area of 64.27 sq. cm. In

order to make a comparison of the rate of transpiration of the two plants, the rate of the latter was calculated on the basis of 100 sq. cm. and of the former on the basis of 64.27 sq. cm. of leaf area, and the corresponding curves plotted. It is to be noted that the rate of transpiration for the plant with roots submerged is lower than either of the calculated rates. Since the actual value probably lies somewhere between the two calculated rates, it seems fair to assume that the difference between the calculated rate and the observed rate for the plant with submerged roots is brought about by submergence.

Later two other seedlings of *Quercus macrocarpa* were added to the first two. Both of the new plants had the roots submerged and were covered in the same way that the others were. Weighings were made at intervals during a period of about three weeks. The results are shown in the following graph (Fig. 3).

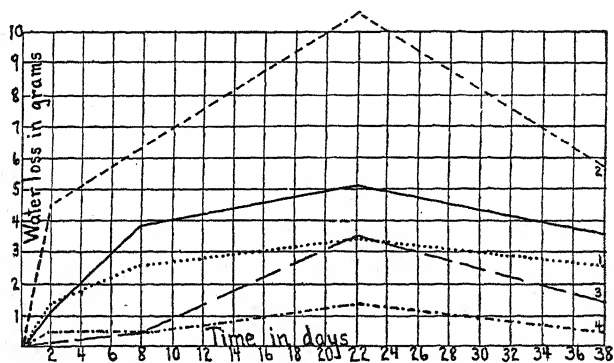


FIG. 3. Comparative rates of transpiration of seedlings of *Quercus macrocarpa* in moist and in submerged soil. No. 2, in moist soil, leaf area 100 sq. cm.; Nos. 1, 3, and 4 in submerged soil. Leaf area: No. 1, 64.27 sq. cm.; No. 3, 48.34 sq. cm.; No. 4, 70.0 sq. cm. The heavy solid line represents the average rate of the three plants based on the same leaf area as No. 2 in moist soil.

The average transpiration is shown for each plant during the intervals between weighings. The plants were of different sizes and with different leaf areas. No. 2, which was grown in moist soil, had a leaf area of 100 sq. cm. The other three plants had the roots submerged. Their leaf areas in square centimetres were as follows: No. 1, 64.27; No. 3, 48.34; No. 4, 70.0. In order to make a comparison, a curve for the average transpiration of the three plants calculated on the basis of 100 sq. cm. of leaf area was drawn. When calculated on equivalent leaf areas the average transpiration of the three plants with roots submerged was much lower than that of plant No. 2, the roots of which were not submerged. Plant No. 1 had the highest rate of transpiration of any of the plants with submerged roots. Even if the transpiration rate of this plant were calculated on the basis of 100 sq. cm., the leaf area of plant No. 2, the calculated rate would

still be considerably lower than that of No. 2. The second trial with *Quercus macrocarpa* thus shows that the rate of transpiration per unit area of leaf surface in plants with roots submerged is much reduced in comparison with the rate of transpiration of plants the roots of which have not been submerged.

*Comparison of the Effect of Swamp Water and Nutrient Solution on the Growth of Plants.*

To compare the effect of swamp water and nutrient solution on the growth of plants under equal conditions of aeration, six pots of corn and six of beans were planted in washed, white quartz sand. The cotyledons of the beans were removed as soon as the first leaves began to expand. The endosperm of the corn was also removed as soon as the first leaf began to unroll. All plants were watered with distilled water for three days. Thereafter three plants each of corn and beans were watered with swamp water and three each with Sachs's culture solution. In four or five days it became evident that the plants watered with swamp water were making less rapid growth than those receiving culture solution. This difference was maintained, and at the end of the experiment the plants watered with culture solution were somewhat larger, stronger, and of darker green colour than those which had been watered with swamp water. The root system was equally well developed in all plants. The difference in the growth of plants watered with swamp water and those watered with culture solution was not very great, and is probably to be attributed to the lack of certain mineral constituents.

*Effect of Philotria on the Gas Content of Water.*

In determining the amount of air dissolved, an apparatus similar to that described in Dennis's translation of Hempel's *Gas Analysis* was used. In the first analyses a flask containing one litre was used, but later this was replaced by one containing two litres. Samples were boiled from three to five minutes. The technical method of analysis was used. Descriptions of this method are to be found in Hempel's *Gas Analysis*, in Lunge's *Technical Gas Analysis*, and in other texts. Duplicate analyses were run in nearly every case. The results were found to check within 0.2 to 0.3 c.c.

The following analyses show the effect of placing *Philotria* in water with good illumination. No. 1 is tap-water which had stood several days in the laboratory; No. 2, tap-water with *Philotria*, analysis made early in the forenoon; No. 3, tap-water with *Philotria*, analysis made in the afternoon after several hours' exposure to good light. Duplicate analyses are given in each case.

TABLE I.

*Gas Content per Litre of Water with and without Philotria.*

No.	Kind of Water.	Carbon Dioxide.	Oxygen.	Nitrogen.
		c.c.	c.c.	c.c.
1	Standing tap	2.8	5.4	12.6
2	"	2.6	5.6	12.4
3	Tap, with <i>Philotria</i> , forenoon	2.8	5.4	12.8
4	" " "	2.6	5.6	12.6
5	" " afternoon	0.6	8.2	13.0
7	" " "	0.8	7.8	12.8

From this it is to be noted that the oxygen and carbon dioxide content are not affected by *Philotria* during the night. During daylight, on the other hand, the carbon dioxide is decreased and the oxygen content is increased. Accordingly the value of *Philotria* as a means of aerating water is established by these analyses. Other analyses, the figures of which are not presented, show that in cloudy weather *Philotria* has little effect on the carbon dioxide or oxygen content. This accounts for the wilting noted during cloudy weather in plants with submerged roots in the experiments with *Impatiens* when *Philotria* was used for aerating the water.

The effect of *Philotria* in water on the carbon dioxide and oxygen content was tried another time. A large glass jar was filled with water and a handful of *Philotria* plants thrown in. The jar was set near a south window so as to receive the best possible light. Analyses were made the next day. The first analysis was at 10.0 a.m., the second at 11.0 a.m., and the third at 11.30 a.m. The results are given in the following table:

TABLE II.

*Gas Content per Litre of Water with Philotria.*

Sample Number.	Time taken.	Carbon Dioxide.	Oxygen.	Nitrogen.
		c.c.	c.c.	c.c.
1	10 a.m.	1.1	6.2	12.2
2	10.30 a.m.	0.8	7.0	12.2
3	11 a.m.	0.5	7.4	12.4

It is to be noted here also, as in Table I, that the presence of *Philotria* in good light causes a decrease in the amount of carbon dioxide and an increase in the amount of oxygen.

*Gas Content of Swamp Water.*

During the summer of 1916, at Hubert, Minnesota, a series of analyses of lake and swamp waters was made. The lake water was from Lake Hubert near the shore in water about two feet deep. Other samples were

taken in the swamp bordering on Mud Lake. Part of the samples were taken in the *Carex-Calamagrostis* associates, and the others in the *Larix-Picea* associates. All swamp samples were taken in areas in which *Sphagnum* and other mosses were abundant. A third series of samples was taken from Henderson's bog, north of Lake Hubert. One sample was taken in the *Carex* associates and the other two below *Sphagnum* in the *Chamaedaphne-Andromeda* associates. The results are presented in the following table:

TABLE III.  
Gas Content per Litre of Water from Various Associates.

Source.	Sample Number.	Carbon Dioxide.	Oxygen.	Nitrogen.
		c.c.	c.c.	c.c.
Hubert Lake	1	1.0	7.6	13.0
"	2	1.4	7.2	12.6
"	3	1.2	7.4	—
"	4	1.2	7.6	12.4
"	5	1.2	7.6	—
"	6	1.0	7.8	12.8
Mud Lake: <i>Carex-Calamagrostis</i>	1	5.8	3.2	16.8
" "	2	6.0	3.0	17.2
" "	3	6.0	3.0	17.2
" <i>Larix-Picea</i>	1	8.4	2.8	17.0
Henderson's bog: <i>Carex</i>	1	8.4	4.6	15.4
" <i>Andromeda</i>	1	9.8	3.9	11.1
" "	2	10.2	3.8	14.8

The field analyses were made with the burette only, the solutions being introduced into the burette. Under the conditions the results are not as accurate as those made in the laboratory with pipettes. Comparison of the results, however, with analyses in the laboratory shows that the errors are not more than twice those of laboratory analyses, and accordingly they may be used in comparing results.

It is to be noted from the figures here given that a marked difference exists between the air content of a lake and that of a swamp. Hubert is a spring-fed lake with cold water and a clean gravelly or sandy bottom. The bottom where the samples were taken was of very coarse gravel or pebbles. The water is well supplied with oxygen. When a lake becomes converted into a swamp, a very evident decrease of oxygen occurs with a marked increase in carbon dioxide content. The oxygen content falls off to half or less that of lake or tap water. This undoubtedly is a very important factor in retarding the growth and activity of roots of plants which do not have air-conducting systems. The sample from the *Carex* zone of Henderson's bog was taken just at the edge where *Carex* was invading the *Castalia* associates. The oxygen content there is somewhat higher than it is in later stages. The high carbon dioxide content is to be explained by the decomposition of organic matter. Any lake which contains a large amount of vegetation is usually found to be low in oxygen and

high in carbon dioxide content. Birge and Juday (2) show that such conditions prevail in Wisconsin lakes with considerable organic matter on the bottom. They also show (l.c., p. 51) that under certain conditions lake water may show a supersaturation of oxygen.

The apparently high nitrogen content is due to the fact that considerable quantities of methane are present in swamps, but the quantity was not determined. In making the analyses the residue after absorption by potash and pyrogallol was regarded as nitrogen. For this reason the nitrogen content of swamp waters always runs too high.

#### DISCUSSION OF RESULTS.

##### *Effect of Root Submergence on Development.*

Experiments with corn, beans, horse bean, and other plants have shown that the roots of land plants are less developed when submerged than when in moist soil. The part of the plant above ground in all the plants named also undergoes a slight reduction in size as compared with the above-ground parts of plants the roots of which are not submerged. If the submergence be prolonged, the more deeply submerged roots die and new ones are developed at or near the surface of the water. Certain plants, such as *Ranunculus abortivus*, *R. sceleratus*, *Cyperus alternifolius*, and *Sagittaria*, when grown with roots submerged showed no retardation in root or shoot development. On the contrary, a better development of roots and of foliage was noted when the roots were submerged than when growing in moist garden soil. Wacker (25, p. 82) in experimenting with the growth of aquatic plants in moist soil obtained similar results. A retardation in the growth of land plants in water has been observed by Sachs (19), Mer (16), Schwartz (22), Wacker (25), Kraus (13), and others. Perseke (17) and Schwartz (22) found that growing land plants with the roots submerged caused a great reduction in the development of root-hairs, and that in many instances no root-hairs were formed.

##### *Effect of Root Submergence on Absorption.*

The absorbing capacity of roots is of vital importance, and very directly and effectively influences the development of the plant. Although it has been repeatedly shown that root submergence causes a retardation in root development, little or nothing has been done, apparently, to determine the effect of root submergence on absorption. In experiments performed by the writer an inhibition in the absorbing capacity of submerged roots is indicated by the etiolation and loss of leaves which soon followed submergence of the roots. This is shown by the graphs (pp. 18-20), which give the comparative rates of transpiration per unit area of leaf surface of plants of *Pelargonium* and seedlings of *Quercus macrocarpa* with roots submerged,

and of others with the roots in moist soil. The results of these experiments indicate that transpiration is greater than absorption, and that the shedding of leaves is to be regarded as a compensation for reduced ability to absorb by a reduction of the transpiring surface.

*The Effect of Aeration on Submerged Roots.*

Arker (1, p. 63) found that the rate of root growth of *Lupinus albus* and *Helianthus annuus* could be increased by passing a current of air through water or soil. He also found that roots of plants in water readily take up oxygen in solution. Kraus (13) shows that the percentage of germination of seeds and the rate of growth of seedlings of land plants in the early stages of development can be greatly increased by supplying oxygen to the water in which the seeds or seedlings are submerged. Dachnowski (5, p. 314) observed a stunting of roots in cultures of wheat, corn, bean, elm, and other plants in bog water. Aeration, he found, remedied the stunting effect.

Hall, Brenchley, and Underwood (11, p. 298), in an experimental study of soil solutions, found that a better root development and far better growth were obtained with silver sand and kaolin than with fine sand, silt, or in water culture. It was suspected that differences in aeration of the roots might be the disturbing factor. The correctness of this supposition was established by growing barley in culture solutions aerated once a day and aerated continuously. The latter gave much the better root development, and also the better growth of leaves and stems.

Hole (12), as a result of the study of the reproduction of *Shorea robusta*, came to the conclusion that the failure of reproduction was due to poor aeration, and states (p. 80) that when proper aeration was provided the injurious effects were quickly dissipated.

Experiments of the writer also show that when aeration is provided the development of roots under submergence is not much, if at all, retarded as compared with the growth of roots in moist soil. No essential difference was to be observed in the behaviour of plants with the roots submerged in swamp water as compared with other plants of the same kind with the roots in tap-water. In either case symptoms of distress were manifested in equal degree in the same period of time, and in either case the plants recovered with equal promptness when air was supplied. In the experiment described on p. 21, only a slight difference in the size and vigour of the plants watered with nutrient solution as compared with those watered with swamp water was observed. The plants in both cases were under the same conditions of aeration, so that differences of size or vigour cannot be ascribed to that cause. The slightly poorer growth noted in the plants watered with swamp water was probably due to a lack of potash or nitrates or both.



*The Amount of Oxygen required for Growth.*

Dehérain and Vesque (6, p. 340) by their experiments established the fact that if the roots were deprived of oxygen the plant itself soon perished. The amount of oxygen necessary for growth is very small according to Wieler, (26) but varies in different plants. He found the maximum growth to take place in *Vicia Faba* with 5-6 per cent. of oxygen. For *Helianthus* 3 per cent. was the optimum amount. With other plants a retardation took place between 14-16 per cent. according to the plant. Vöchting (24, p. 94) found that a reduction of pressure to 3 per cent. or below caused the production of hairs on the roots of potato tubers to cease. Wacker (25, p. 85), in growing seedlings of *Vicia Faba* and *Helianthus annuus* under bell-jars in which the atmospheric pressure had been reduced to one-tenth, found a slight retardation in the root development as compared with plants under full atmospheric pressure. The difference was not great, however, and he could not state with certainty that the retardation was due to the reduction in the amount of oxygen.

Recently Cannon and Free (4, p. 178), in experimenting with various plants, have shown that they behave very differently in their response to a diminution of the oxygen supply to roots. *Coleus* they found to be injured with a small decrease of oxygen below that of normal atmosphere. *Nerium*, on the other hand, is quite resistant to oxygen deprivation and first showed injury after 26 days in an atmosphere of pure nitrogen. They also found that with *Salix* 'entire deprivation of oxygen appeared to be without injurious effect'.

*The Oxygen Content of Various Substrata.*

The oxygen content of the water or other substratum in which the plants are growing is an important factor in determining not only the growth and activity of the roots but of the entire plant. Whether the amount present is sufficient for the plant's needs, and the manner of maintaining or replenishing the supply, are other points to be considered. Boussingault and Lewy (3) and von Fodor (7) found the oxygen content of soils to be somewhat lower than that of atmospheric air. Russell and Appleyard (18) also found the same to be true. In soils under usual conditions there are abundant spaces between soil particles through which air can diffuse, so that a supply of oxygen is available to the roots at all times.

When the soil is saturated, or has water present in excess, the air is driven out of the interstices of the soil. The only oxygen then available is that in solution in the water. The greater part of a unit volume of soil is occupied by soil particles, leaving only a small volume which can be occupied by water. Water, moreover, contains a relatively small amount of oxygen. Accordingly it is evident that much less oxygen is available in



a supersaturated soil than in water alone, and a much greater difference exists between a supersaturated soil and an ordinary soil. This accounts for the observations of Wacker (25, p. 109) that seedlings of *Vicia Faba* and *Lupinus albus* grown in supersaturated soil showed as compared with the amount of growth in moist soil a greater retardation even than when grown in water. He observed, however, that with frequent changing of water the retardation was somewhat less.

Oxygen diffuses slowly through water, so that the supply is not quickly replenished by diffusion alone. The investigations of Kraus (13) show that boiled water in vessels sealed to exclude the air completely prevents the germination of seeds. Boiled water exposed to air after eight days gave a greatly reduced percentage of germination as compared with seeds in unboiled water. The amount of root growth was also much less in the former than in the latter. He also shows that submergence at greater depths decreases the percentage of germination of seeds, which he explains by the slowness of diffusion of oxygen. In open water agitation by wind and convection currents tend to replenish the oxygen supply. These factors are probably more important than diffusion. In supersaturated soil convection currents and surface agitation are not factors, or are of very little importance.

The very small amount of oxygen in the substratum of peat or *Sphagnum* swamps is also to be explained by the difficulty of replenishing the supply from the air. In peat or *Sphagnum* substrata the presence of living *Sphagnum* and of the accumulated remains of dead plants prevents surface agitation of the water and convection currents by which the oxygen content could be maintained. There is also another factor which operates here, and which does not affect the aeration of ordinary soils, or only to a slight extent. This factor is the presence of partly decomposed remains of *Sphagnum* and other plants, which absorb the oxygen and prevent its penetration into the deeper-lying parts of the substratum. Dachnowski (5, p. 372) calls attention to the reducing power of peat, and shows that it is greatest in the central zone and decreases towards the outside.

In comparing the growth of various plants in soil, peat, and *Sphagnum*, with the roots submerged, it was noticed that the roots of plants in peat were usually more retarded in growth than those in soil, and that those in *Sphagnum* showed the greatest retardation. A similar relative reduction in growth of parts above ground was observed. An explanation for this did not at first suggest itself. Later it was found, in the experiment described on p. 21, that only a slight difference in the size and vigour of shoots was noticeable between plants watered with swamp water and those watered with culture solution. The roots, moreover, were equally well developed in all plants. The difference could not have been due to a lack of aeration, since oxygen was available to the roots at all times. The difference was

assumed to be due to the lack of one or more mineral constituents. In view of these facts it seems very probable that the much greater reduction in growth of plants in peat or *Sphagnum*, with roots submerged, was due to a lack of aeration. This conclusion is further confirmed by the fact that analyses show the oxygen content of the water of peat and *Sphagnum* substrata to be much lower than that of lake or tap water. The reducing power of peat and *Sphagnum* also operates to prevent a replenishment of the oxygen supply from the air. It has also been shown that, if aeration is provided, plants grown with the roots submerged in either swamp or tap water show little or no reduction in growth and no essential difference in behaviour as compared with that of plants grown in moist soil.

*The Relation of Roots to the Water-level with Reference  
to the Character of the Plant.*

Plants growing in swamps may be hydrophytic, mesophytic, or xerophytic in character. Many writers regard *Scirpus*, *Equisetum*, *Fucus* and similar plants as bog xerophytes on account of the absence of leaves and general external appearance. The ratio of transpiration to absorption is the important factor in determining the character of a plant. *Scirpus* has been shown by Sampson and Allen (21, p. 49) to be a typical hydrophyte in its rate of transpiration. And as Groom (10) has shown, in the case of *Larix decidua*, some so-called xerophytes transpire more rapidly than some mesophytes. The only plants which are unquestionably bog xerophytes are the Ericads. Gates (8) concludes that winter evaporation is fundamentally responsible for their xerophytic character.

Proximity of location in a swamp does not necessarily mean similarity or identity of conditions either for the roots or for parts above ground. In fact, conditions for different layers are very different, as both Yapp (27) and Sherff (23) have pointed out. The conditions for root growth above and below the water-level are also very different. Hydrophytes with their extensively developed air-conducting systems are able not only to withstand root submergence, but to make better growth than in moderately moist soil. Absorption is not retarded, and however great the water loss from aerial parts it is readily replaced by absorption of water by the roots. Hence there is no need for structural modifications of the aerial organs to prevent water loss.

Plants with roots above the water-level are usually low-growing, and consequently more or less protected from excessive water loss. The absorbing capacity of the roots is little or not at all reduced ordinarily, since good aeration is provided and other factors are not very adverse. Such plants are usually mesophytic in character. However, in dry years or during dry periods of the summer they may be subject to more severe conditions. The upper layers of the substratum become dry, or at least do

not furnish enough available water for plants on account of the great water-retaining power of *Sphagnum*. For this reason plants with roots above water may show more or less severe wilting on the hotter days. If the drought becomes too prolonged or too intense some of these plants may perish.

Plants, such as the Ericads, which are not protected by taller vegetation may be subjected to very great water loss. The roots of these plants are generally not extensive. They ordinarily grow above the water-level or not far below it. They may, however, endure submergence for some time without apparent injury. During the summer the *Sphagnum* on which the bog heaths grow may have but little available water because of the lowering of the water-level and the great ability of *Sphagnum* to retain water, or for other reasons. Therefore, water in sufficient amounts for the needs of the plants might be difficult to obtain. Gates (8, p. 451) states that in such cases 'The xerophytic adaptations . . . materially aid by lessening the demand upon root absorption'. In winter, with continued water loss from the leaves at a time when replacement by absorption is very difficult if not impossible, the xerophytic structures are very valuable in reducing the rate of transpiration.

The character and position of the roots with reference to the water-level has been shown to be directly correlated with the need of aeration. In securing adequate aeration the plant may undergo structural modification by the development of aerenchyma, or the roots may develop above the water-level. In the latter case, however, the amount of water available is involved. If the plants are more or less protected by taller vegetation, or if the substratum does not become too dry, sufficient water is available to replace the loss by transpiration in mesophytes, and they are able to persist. On the other hand, a lowering of the water-level, a cold substratum, or other factors which retard absorption, combined with atmospheric factors which bring about a high rate of transpiration, make it impossible for mesophytes to persist. Only the Ericads with their strongly xerophytic leaf-structures are able to endure such conditions. Accordingly the presence of hydrophytes, mesophytes, and xerophytes in swamps is to be explained by local differences in the habitat. These differences, according to the nature of the adjustment of the roots to the water-level, which influences the amount of water available, affect the ratio of absorption to transpiration and determine the character of the plant.

#### *The Relation of Aeration to Ecesis.*

Many plants are able to establish themselves in habitats with an excess of water. Fruits of *Alnus incana*, *Betula pumila*, *Panicularia americana*, *Rumex britannica*, and *Scirpus cyperinus* germinated, and the seedlings were able to establish themselves on *Sphagnum* when the water-level was kept just at the surface of the *Sphagnum*. Seedlings of *Rumex* were able

to grow when submerged to a depth of one inch. Fruits of *Typha*, *Sagittaria*, and *Alisma* germinate and develop readily under water. It was observed, however, that fruits of *Alisma* and *Sagittaria* in jars of water with clean sand at the bottom failed to develop beyond the early stages of germination. The oxygen and carbon dioxide content of the water was not determined, and consequently it is not known to what extent the concentration of these gases may have been responsible for the failure of the seedlings to develop.

*Carex pseudocyperus*, *Dulichium arundinaceum*, *Ribes* spp., *Salix* spp., and many other plants germinate readily on hummocks of *Sphagnum* or on mounds of peat, but fail to germinate if submerged. Seedlings of *Andromeda glaucophylla*, *Kalmia glauca*, and *Ledum groenlandicum* have been found on *Sphagnum* or peat above the water-level. It seems probable, therefore, that seedlings of these plants are not able to develop under prolonged root submergence. Glück (9) found that the seeds of many plants possessed the ability to germinate and grow under water. Other seeds failed to germinate if covered to a depth of half an inch or less. Kraus (13) has shown that the germination of seeds of land plants may be brought about by aerating the water. Differences are to be observed in the behaviour of various seeds in this respect. Some require less oxygen than others.

The behaviour of roots of seedlings shows the same relation to aeration that is shown by the roots of older plants. Only those plants with well-developed aerenchyma are able to establish themselves in habitats with an excess of water. If the water-level is below the surface slightly, many plants are able to invade and become established. The roots of such plants remain near the surface. Periods of hot, dry weather during the summer, which cause the upper layers of the substratum to become dry, may then result in the death of seedlings with shallow root systems. Thus it is evident that the need for adequate aeration for the germination of seeds and the development of seedlings is an important factor in ecesis. It determines in a large measure the character of the invaders in swamps.

#### CONCLUSIONS.

(1) Roots of land plants do not live under prolonged submergence. The submerged roots die and new ones are developed from the stem at the surface of the water. This occurs whether the plants are grown in loam, peat, or *Sphagnum*.

(2) Land plants grown in peat or *Sphagnum* show an evident reduction in growth of the entire plant when the roots are submerged. This is little or not at all apparent in plants grown in soil with submerged roots.

(3) Reduction in growth of plants in *Sphagnum* with the roots sub-

merged is greater than under similar conditions in peat. This appears to be due for the most part to a greater lack of oxygen in *Sphagnum* than in peat. A similar relation exists between plants in peat and in soil with the roots submerged.

(4) When the water is aerated plants, are able to endure root submergence as long as aeration is maintained. The roots show some retardation in growth, but remain alive.

(5) *Ranunculus abortivus*, *R. sceleratus*, and *Cyperus alternifolius* grown in submerged soil show a greater growth of the entire plant than when grown in moist soil. The ability to grow with the roots submerged is undoubtedly due to the presence of aerenchyma. The reduction of the growth of the plants in moist soil is caused by the inability of the roots to absorb sufficient water under such conditions.

(6) Land plants with submerged roots show more or less pronounced wilting after one to three days. If submergence is prolonged the leaves become yellow and soon fall.

(7) Land plants with submerged roots do not show these effects, or only to a slight extent, in aerated water.

(8) *Philotria* placed in water with good light causes a decrease in carbon dioxide and an increase in oxygen content of the water.

(9) Plants in soil from which oxygen is excluded show wilting, etiolation, and loss of leaves. The effects appear in the same order and same time as in plants with the roots submerged.

(10) Plants with submerged roots show a temporary increase in transpiration, which is soon followed by a sharp decline as compared with the transpiration of plants grown in moist soil.

(11) The reduction in transpiration precedes wilting. It precedes etiolation and loss of leaves by two to four days. This indicates that absorption is reduced below the amount demanded by transpiration.

(12) When aeration is provided, the development of plants is essentially as good with swamp water as with nutrient solution. Plants with swamp water are somewhat smaller. The difference is somewhat more evident when the plants are deprived of reserve food. This indicates that the difference in growth is correlated with the food supply.

(13) The oxygen content of lake water is essentially the same as that of tap or distilled water under similar conditions. The oxygen content of swamp water decreases from the *Carex* stage to the *Chamaedaphne-Andromeda* and *Larix-Picea* stages.

(14) The carbon dioxide content shows a corresponding increase through the same stages. The increase is due to the decomposition of organic matter by which carbon dioxide is liberated.

(15) The adjustment of the roots of swamp plants to the water-level is due to the necessity of securing a sufficient supply of oxygen. This

necessity is met by structural modifications or merely by a change in the level at which the roots develop.

(16) The presence of hydrophytes, mesophytes, and xerophytes in swamps is due to local differences in the habitat. These differences, according to the nature of the adjustment of the roots to the water-level which influences the amount of water available, affect the ratio of absorption to transpiration and determine the character of the plant.

(17) Ecesis is possible for many plants even when submerged. Ecesis can occur only when the oxygen requirements are satisfied.

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# On the Alga-Flora of some Desiccated English Soils : an Important Factor in Soil Biology.

BY

B. MURIEL BRISTOL, M.Sc.

With Plate II and twelve Figures and three Tables in the Text.

## SYNOPSIS.

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## I. INTRODUCTION.

DURING recent years a good deal of attention has been paid to the activities of certain soil-organisms with regard to their significance in the economy of the soil. Proceeding from the wonderful discoveries of Pasteur on bacterial fermentation, Berthelot in 1885 established the fact that the building up of nitrates in the soil is due to the activities of certain micro-organisms. This discovery led to wider experiments in all directions, and especially to the investigations of Hellriegel and Wilfarth in connexion with the nitrogen supply of leguminous plants, and of Winogradsky and Beijerinck on the work of nitrogen-fixing bacteria in the soil.

These activities mark the beginning of a new era in biology, and have led to the establishment of a special branch of the science, soil biology, dealing with problems of interest not only to biologists but also to practical agriculturists. The activities of the nitrogen-bacteria of the soil are now well established, while those of the attendant soil-protozoa have already received a considerable amount of attention; and the pathogenic effect on higher plants of nematodes, myxomycetes, bacteria, and fungi occurring in soils is studied as a special branch of plant pathology. The algae of soils have, on the contrary, possibly on account of their supposed lack of importance as factors in the economy of the soil, been almost entirely over-

looked, and, in this country at any rate, no systematic work has so far been done in connexion with them. This is all the more extraordinary since the researches of Kossowitsch, Bouilhac, Giustiniani, Schloesing, Laurent, and others clearly indicate that there is an intimate connexion in the soil between algae and the nitrogen-fixing bacteria, and that algae probably play quite a prominent part in the activities carried on by these organisms.

In 1910 Esmarch<sup>1</sup> published a paper on the blue-green algae of the soils of the German African colonies, and as a result of his experiments he concluded that cultivated soils are throughout richer in blue-green algae than are uncultivated soils, and that in cultivated soils algae occur not only in the surface but also in the lower layers. As a consequence of this work he further undertook an investigation of the distribution of the blue-green algae in different soils of Schleswig-Holstein<sup>2</sup> for the purpose of finding out not only what species are present in different kinds of soil, but also the depths to which they may be found and the possible reasons for their occurrence in such positions.

Meanwhile, in 1912, Robbins<sup>3</sup> published a paper on the algae in some Colorado soils, in which he described twenty-one different species obtained from cultures of twenty-two different soils, and discussed their probable connexion with the accumulation of unprecedented quantities of nitrates in certain soils in Colorado.

Petersen's work<sup>4</sup> on the sub-aerial algae of Denmark is a valuable contribution to our knowledge of soil-algae, especially in so far as it concerns soil-diatoms; but his investigations of the green algae appear to have been confined to forms visible on collection to the naked eye, and his lists can therefore scarcely be regarded as exhaustive in this direction. His omission of all reference to blue-green algae also makes it difficult to realize a true conception of the nature and extent of the algal flora of the soils.

In the course of an investigation of soil-protozoa, Dr. T. Goodey and Dr. H. B. Hutchinson recently obtained certain algal forms from old stored soils and sent them to Professor G. S. West for identification. This was impossible at first, but after an investigation extending over several months Professor West was enabled to state that spores of *Nodularia Harveyana*, (Thwaites) Thuret, are able to retain their vitality for a period of sixty-six years.<sup>5</sup> This discovery led him to suggest that the study of a number of dried soils by means of cultures might be a profitable one in ascertaining

<sup>1</sup> Esmarch, F.: Beitrag zur Cyanophyceen-Flora unserer Kolonien. Jahrbuch der Hamburgischen wissenschaftl. Anstalten, xxviii, 3, Beiheft, S. 62-82, 1910.

<sup>2</sup> Esmarch, F.: Untersuchungen über die Verbreitung der Cyanophyceen auf und in verschiedenen Boden. Hedwigia, Band lv, Heft 4-5, September 1914.

<sup>3</sup> Robbins, W. W.: Algae in some Colorado Soils. Bulletin 184, Agr. Exp. Sta. Colorado, June, 1912.

<sup>4</sup> Petersen, J. B.: Danske aerofile alger. D. Kgl. Danske Vidensk. Selsk. Skrifter, 7 Række, Naturv. og Mathem., Afd. xii, 7, 1915.

<sup>5</sup> Vide West, G. S.: Algae. I Camb. Bot. Handbooks, p. 28, 1916.

whether in this country there exist many algae that are sufficiently resistant to desiccation to be able to persist in the soil during any period of drought that might naturally occur. The investigation was begun in September, 1915, when a collection was made of small samples of cultivated soils from different parts of the country. The samples were taken from arable land or from old gardens, in such places as appeared to be destitute of vegetation, and were then spread out to dry gradually in a warm room for at least a month, care being taken to prevent foreign infection of the soils by covering them with sterile paper. This preliminary drying of the soils served to kill off any algae present that might be unable to resist prolonged desiccation and to induce any more resistant ones to enter into a resting state. When completely dry, the soil-samples were placed in small tin boxes for storage; by this time the soil in almost every case had crumbled into a fairly fine powder.

## II. CULTURAL METHODS.

Three cultures of each sample were made, with the exception of Nos. 5 and 35-40, of which there were only two. The cultures were set up in very carefully sterilized<sup>1</sup> vessels which comprised glass boxes and small conical flasks or wide-mouthed bottles closed with plugs of cotton-wool. Into each of the sterilized culture-vessels a sterilized culture-medium was introduced to a depth of about half an inch, and into this a few grammes of the soil to be examined were introduced by means of a sterilized spatula. The vessels were closed and placed under glass cases in a north window and left for some months to develop.

The culture-solution most generally used was an aqueous mineral-salt solution having the following composition: 1 gram.  $\text{KH}_2\text{PO}_4$ , 1 gram.  $\text{NaNO}_3$ , 0.3 gram.  $\text{MgSO}_4$ , 0.1 gram.  $\text{CaCl}_2$ , 0.1 gram.  $\text{NaCl}$ , a trace of  $\text{FeCl}_3$ , 1,000 c.c. distilled water.

But for the sake of comparison certain of the cultures were made with a solution diluted with distilled water to half the above strength, and others with sterilized rain-water. Evaporation from the surface of the cultures took place only slowly, and it was found sufficient, in order to keep them moist, to water them at the end of about six months with sterilized distilled water or with rain-water; in one or two cases where the cultures were assuming a brownish colour diluted mineral-salt solution was added.

The first signs of growth were observed in the cultures at about the end of November, when a thin white scum gradually appeared on the surface of the liquid, and was found on examination to consist of bacteria. About six weeks later the scum began to assume a green tinge of colour, and small tufts of green filaments were observed to be growing from the soil at the

<sup>1</sup> All the culture-vessels were heated on three separate occasions to a temperature of about 120° C., and kept at that temperature for three hours on each occasion. The culture-media were heated in a steam sterilizer for about three hours on each of three occasions.

bottom of the culture. Owing to the preliminary drying of the soil the cultures for some time contained only developmental stages of algae, and a considerable period elapsed before their identification could be accomplished; but it was easily seen that the green tufts consisted of filaments of moss protonema. At first only green algae and diatoms appeared to be growing in the cultures, forming a stratum on the surface of the liquid and on the sides of the culture-vessel, while the whole liquid assumed a light green colour owing to the presence of free floating unicells. Later, however, patches of blue-green algae began to appear on the surface of the soil and on the glass sides of the vessel below the surface of the water.

Esmarch and Robbins in their investigations adopted rather different methods of culture from the above, but in neither case did the algae grow under quite natural conditions. Esmarch used Petri dishes about 2 cm. deep, in which he kept thoroughly moistened a layer of the soil to be examined; on the surface of the soil he placed a piece of chemically pure filter-paper which was kept in close contact with the soil by constantly smoothing it out with a sterilized iron spatula. He found that the blue-green algae in the soil germinated and grew towards the light through the pores of the filter-paper and produced strata of various forms on the upper surface of the paper. He observed that green algae and moss protonema also grew occasionally, but very rarely in sufficient quantity to affect the culture to any extent. His result is thus quite different from that obtained in the present work, and unless the prolific growth of green algae in the cultures of English soils is to be regarded as a peculiar characteristic of the soils of this country, it appears rather as though the presence of the filter-paper in Esmarch's cultures tended to suppress the growth of green algae or at any rate to prevent their penetration to the surface of the culture. Of diatoms Esmarch makes no mention, and it is probable that if they were present in the soils they never penetrated the filter-paper and so were completely overlooked.

Robbins for his cultures used half-litre flasks filled to their greatest diameter with washed and sterilized moist ground quartz, on the surface of which was distributed as evenly as possible 10 grm. of the soil to be examined suspended in 25 c.c. of distilled water. The flasks were tilted to one side so as to provide both a moist sand and a free water surface for the algae to grow on. In this way the algae were set to grow on a medium the physical qualities of which were quite different from those of the original soil in that all organic substance had been extracted from the quartz before use; also the addition merely of distilled water without any dissolved mineral salts must have caused the algae to be subjected to a somewhat inadequate food supply, in view of the large quantity of insoluble sand present in proportion to the small amount of soluble salts in the inoculated soil.

It is conspicuous that of twenty-one different species obtained by Robbins from the Colorado soils only one belonged to the diatoms and one to the green algae; the rest all belonged to the blue-green algae.

Petersen, on the other hand, found in cultivated Danish soils no less than twenty different species of diatoms and eighteen different species of green algae, a result very similar to that obtained in the present work, hence it appears from Robbins's record that the Colorado soils may be peculiar in this respect.

A comparison of the results obtained with the different culture-solutions used in the present work showed that there was very little difference between the cultures. Germination of the green algae and diatoms took place rather more quickly in the more dilute mineral-salt solution and in rain-water, while the development of the blue-green algae was on the whole more vigorous in the stronger mineral-salt solution; rain-water appeared as a rule to favour especially the development of the green algae. In every case, however, the final results were identical, cultures of the same soil giving always the same record of species present, whichever culture-medium might be used.

### III. RESULTS OF CULTURAL EXPERIMENTS.

A great deal of difficulty was experienced in identifying the algae found in the cultures for various reasons. In the first place, the preliminary treatment of the soils was such as to preclude the possibility of the presence of any algae except in a resting condition in the initial stages of the cultures. The length of time taken for the germination of these resting forms varied in individual species, and for some months the cultures contained largely developmental stages which it was impossible to identify with any degree of certainty. Again, the somewhat abnormal conditions of excessive moisture under which the algae were growing tended to produce forms which in some cases were rather different from those of typical species already described, and it was necessary to decide whether such variations were the result of these conditions or whether they might perhaps characterize new species or varieties. Further, it was necessary in many cases to follow out the complete life-history of the alga before coming to any decision, and for this purpose it was sometimes necessary to prepare sub-cultures in order to separate the alga in question from the other species in the culture. This was especially the case for the species *Chlorococcum humicola*, (Naeg.) Rabenh., the complete isolation of which was effected by picking out individual cells under the microscope with fine glass capillary tubes and placing them in hanging-drop cultures in a damp chamber. It was found that the algae rarely lived very long in these conditions, but sufficient evidence was obtained to establish the course of the life-history of the species.

In identifying the diatoms various methods were attempted, but the

dimensions of the species present were so small that only one method proved really satisfactory. In this the material to be examined was placed in water on a glass slide and broken up into very small pieces with a pair of needles; the water was then allowed to evaporate slowly in the air, the slide being covered with a glass lid to prevent the access of dust. The material thus became dried on to the slide and could be subjected to further treatment with little risk of loss. After 24 hours' drying, the slide was placed in a boiling-tube containing strong nitric acid and heated to remove all organic matter; after careful washing in water it was transferred through 95 per cent. and absolute alcohol to xylol, and the film of diatoms still remaining attached to the slide was then mounted in Canada balsam. Owing to the minute size of most of these soil-diatoms the markings on the walls were extremely difficult to make out, even under a magnification of 1,435, hence later preparations were made using dammar lac as a mounting medium in order to secure a better definition of the walls.

The accompanying Tables I, II, and III give full particulars of the experimental details and serve for a comparison of the results obtained from the different cultures. An examination of these results leads to several interesting conclusions in relation to the distribution of algae in the soils examined; and in this connexion it may be mentioned that though taxonomically it belongs to a different group of plants, the protonema of mosses has been included with the soil-algae in considering the possible economic significance of the microscopic green plants of the soil, since its form and mode of growth render it physiologically equivalent to them.

It is easily seen that in a large majority of the soil-samples there is a central group of algae comprising most or all of the following species *Hantzschia amphioxys*, *Trochiscia aspera*, *Chlorococcum humicola*, *Bumilleria exilis*, and less often *Ulothrix subtilis*, var. *variabilis*, together with moss protonema. These species thus appear to form the basis of an extensive ecological plant-formation in which, by the inclusion of other typically terrestrial but less widely spread species, smaller plant-associations can be recognized. In certain of the soils associations consisting very largely of diatoms are present, the species most generally found being *Navicula nutica* formae, *N. Atomus*, *N. contenta*, var. *biceps*, *N. Balfouriana*, *N. Brebissonii*, var. *diminuta*, *N. Pupula*, and to a lesser degree *N. borealis*. It is conspicuous that with the exception of *Navicula borealis*, *Hantzschia amphioxys*, and *Nitzschia obtusa*, var. *scalpelliformis*, all the diatoms found in these soils are of very minute size, and it is no doubt this characteristic which enables them to withstand the conditions of drought to which the organisms of the soil are liable to be subjected; since, as has been pointed out by Hedlund,<sup>1</sup> small organisms seem to be better able to resist desiccation than are larger ones. Even in the cases of the larger species

<sup>1</sup> Hedlund, T.: Till frågan om växternas frosthärdighet. Botanisker Notiser, Lund, 1913.

mentioned the forms met with from these soils are small in comparison with other forms and varieties of the same species occurring in aquatic habitats. These facts are also true for the diatoms described by Petersen from the Danish soils. He suggests that since the species belong to the pennate diatoms they are further adapted to their mode of life by their power of locomotion, so that they are enabled to move in times of drought to the moister layers of the soil.

In a recent letter to Professor G. S. West, Dr. A. Mann, of the U.S. Dept. of Agriculture, has suggested that the exceedingly small size of these diatoms, especially of *Navicula contenta*, var. *biceps*, is due to long starvation under unfavourable biological conditions, and that they are merely forms of some of the larger species which have undergone not only reduction in size but also suppression in the sharpness of their markings; he suggests that *Navicula contenta* is either a dwarf variety of, or closely related to, *Navicula gibba*, Ehrenb., or some such variable form. These suggestions, however, do not seem likely, since in the cultures under present investigation, where a plentiful supply of water and of mineral salts was always available, no change in the form or size of the diatoms was observable even at the end of three and a half years, but the species remained true to type; and besides this the soil can scarcely be regarded as a medium in which diatoms are subjected to prolonged starvation, especially in a country like England, where there is a fairly adequate rainfall, and where, even in the absence of rain, there is usually a copious dew. The typical form of *Navicula contenta*, var. *biceps*, has also been recorded by Petersen from Danish soils, while specimens of the same variety growing on the leaves of trees at Watten Waven, Dominica, W. Indies,<sup>1</sup> were found on comparison to show characters identical with those of the soil form. The ability of this diatom to grow in so apparently unpropitious a habitat as the leaves of trees is possibly the result of the extreme moisture of the atmosphere in Dominica, and the diatoms are probably able to obtain all the nourishment they require from this source.

It seems most correct, then, to regard the soil-diatoms as independent species or varieties which by their small size have been enabled to establish themselves in a habitat which would be unable to support life and growth in the larger species.

In the soils examined in this work blue-green algae are less universally present than are diatoms or green algae, and the species found appear to be more local in occurrence. There can, however, be traced in a number of soils an association between the three species *Phormidium tenue*, *P. autumnale*, and *Plectonema Battersii*, at least two of the three species having been found together in no less than sixteen soils, while all three occur in

<sup>1</sup> West, W. and G. S.: A Further Contrib. to Freshw. Algae of W. Indies. Journ. Linn. Soc., Bot., xxxiv, 1899, p. 291.

TABLE I.

No. of Sort	Locality.	Kind of Soil. R. = Arable. G. = Garden. P. = Peaty.	Date of Collection of Soil.	Date of Inoculation of Culture.	Period of Growth, in Weeks (Approx.).	BACILLARIAE.
1	Kettering A.	R	ix. 15.	x. 15.	4 1/2	<i>Nocardia horvathii</i> , Ehr.
2	" B.	R	ix. 15.	x. 15.	4 1/2	<i>Nar. balsamiana</i> , Grun.
3	" C.	G	ix. 15.	x. 15.	4	<i>Nar. brethissonii</i> , Kuetz., var. <i>diminuta</i> , Van H.
4	" D.	G	ix. 15.	x. 15.	4	<i>Nar. intermedia</i> , Lagerst.
35	Kew A.	G	ix. 15.	x. 15.	4	<i>Nar. elliptica</i> , Kuetz., var. <i>gella</i> , Naeg.
36	" B.	G	ix. 15.	x. 15.	4	<i>Nar. elliptica</i> , Kuetz., var. <i>minima</i> , Van H.
37	" C.	G	ix. 15.	x. 15.	4	<i>Nar. terricola</i> , n. sp.
38	" D.	G	ix. 15.	x. 15.	4	<i>Nar. mutica</i> , Kuetz.
39	" E.	G	ix. 15.	x. 15.	4	<i>Nar. mutica</i> , var. <i>quinguenodis</i> , Del.
40	Chiswick.	G	ix. 15.	x. 15.	4	<i>Nar. Pupula</i> , Kuetz.
8	Edgbaston A.	G	ix. 15.	x. 15.	4	<i>Nar. Pupula</i> , var. <i>undulata</i> , nov. var.
9	" B.	G	ix. 15.	x. 15.	4	<i>Nar. exilisissima</i> , Grun.
10	" C.	G	ix. 15.	x. 15.	4	<i>Nar. Atomus</i> , Naeg.
11	" D.	G	ix. 15.	x. 15.	4	<i>Nar. contenta</i> , Grun., var. <i>biceps</i> , Del.
12	" E.	G	ix. 15.	x. 15.	4	<i>Nar. (Lophonectis) hyalina</i> , Donk., var. <i>minima</i> , n. var.
13	" F.	G	ix. 15.	x. 15.	4	<i>Nantusia amphioxys</i> , (Ehr.) Grun.
14	Summerfield, Birmingham.	G	ix. 15.	x. 15.	4	<i>Nitroschia obtusa</i> , W. Sm., var. <i>scalloformis</i> , Grun.
15	Warley, Birmingham.	G	ix. 15.	x. 15.	4	<i>Nit. (Pala), (Kuetz.) W. Sm.</i>
16	" "	G	ix. 15.	x. 15.	4	<i>Nit. Pala</i> , var. <i>ponticola</i> , Grun.
17	Harborne A.	R	ix. 15.	x. 15.	4	<i>Nit. inconspicua</i> , Grun.
18	" "	R	ix. 15.	x. 15.	4	
19	" "	R	ix. 15.	x. 15.	4	
20	" "	R	ix. 15.	x. 15.	4	
21	" "	R	ix. 15.	x. 15.	4	
22	" "	R	ix. 15.	x. 15.	4	



23	Northfield, Wores.	R.	14. x. 15.	4. xii. 15.	1. 15.
27	Chadwick, Wores.	R.	20. x. 15.	4. xii. 15.	2. 15.
17	West Bromwich, Staffs.	G.	14. x. 15.	30. xi. 15.	7. 15.
11	Gospel End, Staffs.	G.	7. x. 15.	17. xi. 15.	6. 15.
12	Sedgley, Staffs. A.	R.	7. x. 15.	17. xi. 15.	8. 15.
13	" B.	R.	7. x. 15.	30. xi. 15.	8. 15.
14	Baggeridge, Staffs. A.	R.	7. x. 15.	30. xi. 15.	8. 15.
15	" B.	G.	7. x. 15.	30. xi. 15.	8. 15.
28	Himley, Staffs.	G.	20. x. 15.	4. xii. 15.	6. 15.
42	Tettenhall, Staffs.	R.	27. x. 15.	15. iv. 16.	24. 15.
16	Gravelley Hill, Birmingham.	G.	8. x. 15.	30. xi. 15.	8. 15.
43	Sutton Coldfield.	P.	18. ix. 15.	4. xii. 15.	10. 15.
29	Tisbury, Wilts. A.	G.	20. x. 15.	4. xii. 15.	6. 15.
30	" B.	R.	20. x. 15.	4. xii. 15.	6. 15.
31	" C.	R.	20. x. 15.	4. xii. 15.	6. 15.
32	" D.	R.	20. x. 15.	4. xii. 15.	6. 15.
33	" E.	G.	20. x. 15.	4. xii. 15.	6. 15.
34	" F.	G.	20. x. 15.	4. xii. 15.	6. 15.
45	Hay, Herefordshire.	G.	14. x. 15.	15. iv. 16.	26. 15.
48	Selby, Yorks. A.	G.	? xii. 15.	15. iv. 16.	16. 15.
49	" E.	R.	? xii. 15.	15. iv. 16.	16. 15.

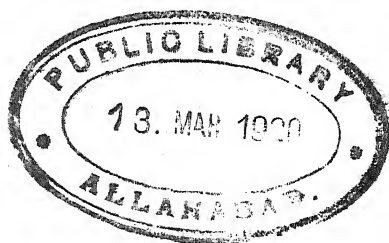


TABLE II.

No. of Culture.	No. of Soil.	Locality.	Kind of Soil. R. = Arable G. = Garden. P. = Peaty.	Period of Growth, in Weeks (Approx.).	<i>Noctac muscorum</i> , Kuetz.	<i>N. commune</i> , Vaucher.	<i>N. spharoides</i> , Kuetz.	<i>N. minutum</i> , Desmaz.	<i>N. foliaceum</i> , Mongeot.	<i>Anabaena varabilis</i> , Kuetz.	<i>A. inaequalis</i> , (Kuetz.) Born. et Flah. forma.	<i>A. sphaerica</i> , Born. et Flah. forma.	<i>A. oscillarioides</i> , Bory, var. <i>terrestris</i> , Bristol, forma <i>minor</i> .	<i>Anabaena</i> sp.	<i>Notularia Harveyana</i> , (Thwaites) Thuret.	<i>Cylindrosporum majus</i> , Kuetz.	<i>C. licheniforme</i> , (Bory) Kuetz.	<i>C. muscicola</i> , Kuetz.	<i>C. warwickum</i> , Lerm., forma <i>tenue</i> .	<i>Plectonema Battersii</i> , Gomont.	<i>Sygonema javanicum</i> , (Kuetz.) Bornet.	<i>? Sygonema</i> sp., juvenile form.	<i>Lynghya Kuetzingii</i> , Schmide.	<i>Rhormidium tenue</i> , (Menegh.) Gomont.	<i>Ph. Bohneri</i> , Schmide.	<i>Ph. laetiusculum</i> , (Ag.) Gomont.	<i>Ph. autumnale</i> , (Ag.) Gomont.	Total No. of Species.	
15	1	Kettering A.	R.	4½	x										x					x									1
16	2	" B.	R.	4½																									2
17	3	" C.	R.	4½																									3
18	4	" D.	R.	4																									4
20	35	Kew A.	G.	4																									5
21	36	" B.	G.	4																									6
22	37	" C.	G.	4																									7
23	38	" D.	G.	4																									8
24	39	" E.	G.	4																									9
25	40	Chiswick.	G.	4																									10
28	8	Edgaston A.	G.	4																									11
29	9	" B.	G.	4																									12
30	10	" C.	G.	4																									13
33	18	" D.	R.	6½																									14
38	24	" E.	R.	7																									15
44	24	" F.	G.	7																									16
45	25	"	G.	7																									17
47	27	Summerfield, Birmingham.	G.	7																									18
48	26	Warley, Birmingham.	G.	7																									19
50	20	" B.	G.	7																									20
41	21	" C.	R.	7																									21
42	22	"	R.	7																									22
43	23	California, Birmingham A.	R.	7																									23
44	24	" B.	R.	7																									24



TABLE III.

[illegible]



seven of them. This association is not universal, however, since each of the three algae has been found in soils from which both of the others were absent. It is noticeable that, of the soils examined, those that are rich in blue-green algae contain only few species of diatoms, and vice versa, though certain exceptions to this rule occur. It seems from the results of the cultures that diatoms occur most frequently in soils from old gardens, whereas the blue-green algae are more characteristic of arable soils; the green algae, on the other hand, are distributed universally.

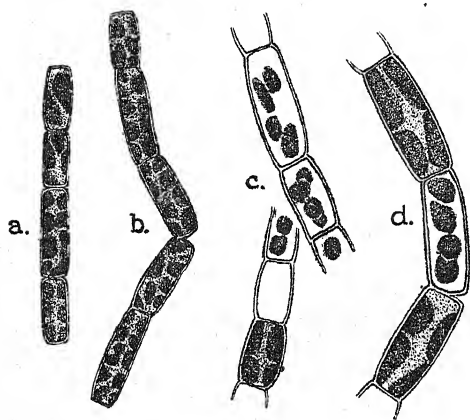
It is a feature of the cultures that certain species of algae which are regarded as typical and commonly occurring soil-forms either are completely absent from them or else occur only rarely. It is possible that the drying of the soil-samples may be responsible for this, for in a number of these

species no record has ever been made of their ability to form resting spores; the degree of desiccation produced in the soils by their preliminary treatment considerably exceeded that likely to occur under natural conditions, and would render the survival of vegetative filaments of the algae quite impossible. An interesting example of this is found in the species *Nostoc commune*, which has been obtained only in the cultures of three arable soils from Tisbury (Nos. 51, 52, and 53). Up to the present time

spores of this species have been unknown, and can therefore be produced only rarely, but in the forms found in these cultures spore-formation has been observed (Text-fig. 4), and it is probably only owing to their production during the first drying of the soils that the species has survived in these particular samples.

Similarly, *Nostoc minutum*, Desmaz, has not previously been observed to produce resting spores, yet in cultures 53, A, B, and C, of an arable soil from Tisbury, the species was not only obtained after a period of desiccation lasting for more than six weeks, but was also observed to produce spores in the culture (Text-fig. 6).

On the other hand, certain species have appeared in the cultures which were entirely unexpected. The most interesting of these is perhaps *Bumilleria exilis* (Text-fig. 1), which was described by Klebs in 1896 from cultures of a loamy soil, and has not since been recorded from any other

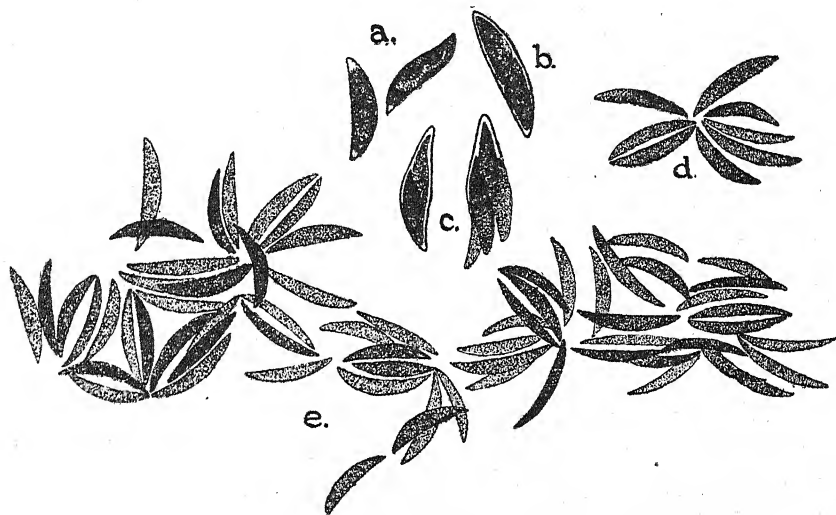


TEXT-FIG. 1. *Bumilleria exilis*, Klebs. a. and b., vegetative filaments showing variable number of chloroplasts,  $\times 825$ ; c. and d., filaments showing stages in formation of zoogonidia,  $\times 1435$ .

habitat. In this country no record of the species has ever been made, hence its appearance from no less than forty of the soil-samples under investigation in the present work is all the more remarkable.

Another interesting record is the finding of *Chlorochytrium paradoxum* in three of the soils. In the Himley (47) and Tisbury, A (49) samples the alga was present in considerable quantity, while in the Sutton Coldfield (48) sample a few isolated specimens only were observed; the cells were embedded in a stratum formed of blue-green algae, but even this association is a great change from the endophytic habit that has previously been described for the species.

*Ankistrodesmus falcatus* has hitherto been regarded as a purely aquatic species, but relatively large colonies of very characteristic form (Text-fig. 2)



TEXT-FIG. 2. *Ankistrodesmus falcatus*, (Corda) Ralfs, forma *terrestris*. a., vegetative cells; b. and c., stages in formation of autospores; d., small colony of eight autospores; e., large colony showing characteristic arrangement of cells. Figs. a.-c.  $\times 1435$ , Figs. d. and e.  $\times 825$ .

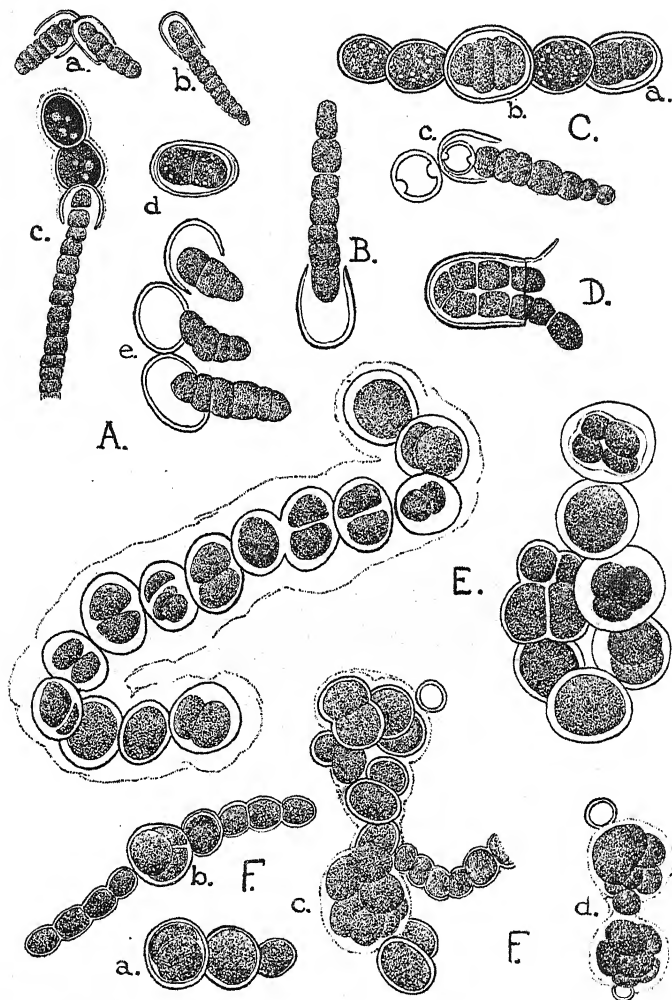
appeared in the cultures of the Warley soil, and that after desiccation for more than five weeks. Hence it appears that this species possesses powers of resisting desiccation that seem almost incredible in an alga of such a size and form. It is probable that in the majority of cases *Ankistrodesmus falcatus* would be unable to withstand desiccation in this manner; hence, in order to emphasize the unusual habitat and extraordinary powers of resistance of the alga in these cultures, it seems advisable to regard it as a special form of the species under the name *A. falcatus*, forma *terrestris*.

The described species of *Gongrosira* also are aquatic in habit; the form that has appeared from three widely separated soil-samples, while obviously belonging to this genus, differs from those already described in



several important particulars and is evidently a new species probably growing only in soils.

Among the blue-green algae, the occurrence of *Plectonema Battersii* in



TEXT-FIG. 3. Germination of spores of some Myxophyceae. A. *Nodularia Harveyana*, (Thwaites) Thuret; a.-c. from old stored soil, 1846; d. and e. from recent soil. B. *Anabaena oscillarioides*, Bory, forma. C. *Anabaena sphaerica*, Born. et Flah.; a.-c., successive stages in germination. D. *Cylindrospermum licheniforme*, (Bory) Kuetz. E. *Nostoc sphaeroides*, Kuetz. F. *Nostoc muscorum*, Kuetz.; a.-d., successive stages in germination. All figs.  $\times 825$ .

thirteen of the soil-samples is interesting, since it has previously been described only as a marine aquatic species.

Owing to the conditions under which the experiments have been carried out, a number of observations have been made of new or interesting

stages in the development and life-history of a number of the species found. This is especially true of the germination and development of some of the blue-green algae, which in some cases have seemed somewhat obscure. In these cultures two quite different methods of germination were observed, a direct germination into a more or less typical vegetative plant and germination into what can only be regarded as a juvenile form bearing none of the characters of the adult plant. The former appears to be by far the more general method of germination and may be subject to slight variation in details (Text-fig. 3, A-D); the latter is largely found in the genus *Nostoc* (Text-fig. 3, E and F).

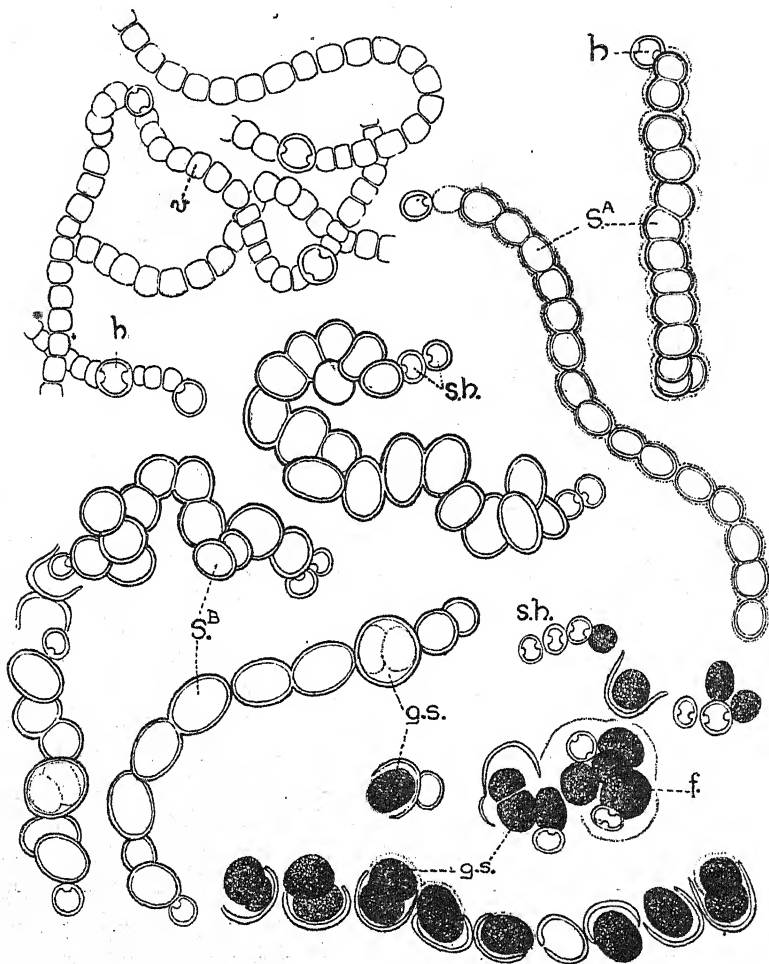
In *Nodularia Harveyana* (A) and in *Anabaena oscillarioides* forma (B) a single transverse division of the protoplast of the spore appears to take place before the wall is ruptured; some part of the wall then becomes diffuent and the free end of the filament is gradually protruded through the aperture as a result of the succeeding divisions of the young cells. The end of the filament remaining within the spore in *Nodularia Harveyana* may remain in this position until the filament has attained a length of more than twenty cells, though in other cases short filaments of not more than six cells have been observed quite free from the spores which produced them. The young filaments soon become broken up into hormogones terminated by heterocysts. The spores were frequently observed to germinate in the cultures while still in series, without the interposition of a resting period after formation.

In *Anabaena sphaerica* (C) the spores were also observed to germinate immediately after formation and while still in series, but in this case at least four transverse divisions of the protoplast were effected before the cell-wall was burst open. Later the end of the spore became gelatinous and the young filament was protruded through the aperture. The basal cell soon became converted into a heterocyst and remained for a considerable time within the old spore-cavity, while the free end extended farther and farther from the spore as a result of succeeding divisions of the young cells.

In *Cylindrospermum licheniforme* (D) the end of the spore was observed to open as a lid to permit the extrusion of the young filament. Several divisions of the protoplast took place before the wall was ruptured, and it sometimes, though not always, happened that a doubling of the filament took place so that both ends of it were protruded at once through the terminal aperture, as shown in the figure.

In *Nostoc commune* (Text-fig. 4) a rejuvenescence of the protoplasm appeared to take place inside the spore, with the result that the spore-wall burst and the contents were extruded either immediately or after a single division within a mucilaginous envelope formed from the inner layers of the spore-wall. In most cases this aperture was produced by a dissolution of part of the wall, but in a few cases a circular portion of the wall was

observed to open as a lid. Within the mucilaginous investment a few divisions were observed to take place, resulting in the formation of a short irregular filament usually terminated at each end by a heterocyst. Heterocysts arranged in short series of two or three were often observed at a slightly later stage of development. It is probable that the cells of the

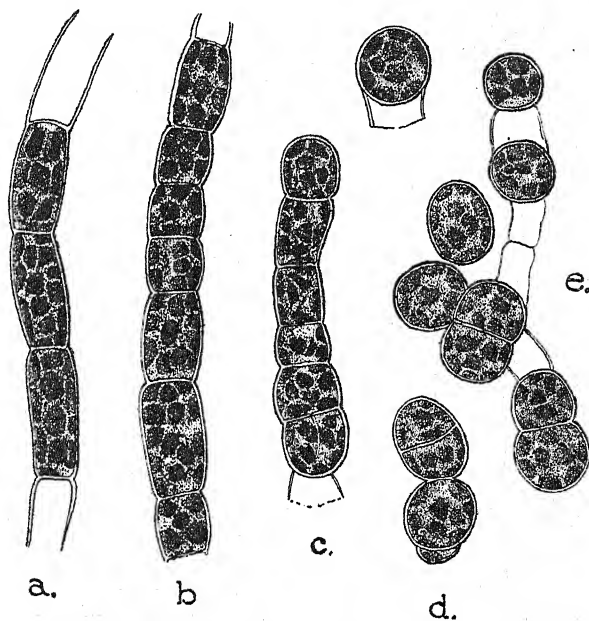


TEXT-FIG. 4. *Nostoc commune*, Vaucher, from arable soil, Tisbury, Wilts. *h.*, heterocyst; *s.h.*, seriate heterocysts; *S.A.*, spores of form A; *S.B.*, spores of form B; *g.s.*, germinating spore of form B; *f.*, young filament from germinated spore; *v.*, vegetative cells of form A. All figs.  $\times 825$ .

young filaments divide irregularly after a time, since numbers of small colonies were observed in the cultures comparable to the juvenile forms described below.

In other species of *Nostoc* direct germination into a short filament, as observed in *N. commune*, did not usually take place, and juvenile forms were

frequently found. The spores germinated in the cultures either after a period of rest or almost immediately after formation. The contents of the spore were observed to become much increased in volume (Text-fig. 3, F, *a* and *b*) and the entire spore-wall to be gradually converted into mucilage. Within this mucilaginous investment the protoplast began to divide in all directions of space, producing a more or less spherical cluster of cells in which no trace of a filamentous arrangement could be detected. These cells continued to divide until a large irregular mass of closely clustered cells was produced which bore no specific characters at all. Heterocysts were usually



TEXT-FIG. 5. *Tribonema bombycinum*, (Ag.) Derb. and Sol. *a.*, typical vegetative filament with walls of end cells broken into H-pieces; *b.* and *c.*, somewhat irregular filaments probably produced as a result of cultural conditions; *d.*, breaking up of filament into aplanospores; *e.*, formation of aplanospores within cells of filament and liberation by splitting of mother-cell wall.  $\times 825$ .

completely absent except in quite young colonies, where a single heterocyst was often observed at the surface of the cell-cluster (Text-fig. 3, F, *d*). The mucilaginous investment usually became firm on the outside and of a light brown colour, and remained permanent. In some cases in the cultures the alga remained in this condition for a considerable time, and sometimes the conditions were such that the cells at this stage became converted directly into resting spores without having developed into the adult stage at all, but this was probably quite abnormal and due entirely to the cultural conditions. In the majority of cases, sooner or later the juvenile form became gradually transformed into the adult stage with definite

filaments producing heterocysts and spores in the normal manner, and in this stage it was possible to identify the species more or less accurately. In Text-fig. 4<sup>3</sup>, E and F, are depicted the initial stages of this mode of germination in two species, *Nostoc sphaeroides*, Kuetz., and *N. muscorum*, Kuetz.

Among the Chlorophyceae the most interesting record of this kind is the formation by *Tribonema bombycinum* of a somewhat unusual type of aplanospore. The filaments of this species appear to be somewhat sensitive to cultural conditions, as can be seen in Text-fig. 5, *b* and *c*, and in the cultures of one soil were observed to be converted into one- or two-celled fragments which rounded themselves off from the rest. In some cases these fragments appeared to be produced simply by the rounding off and breaking away of the cells from one another (Text-fig. 5, *d*), and the wall of the original cell was incorporated with that of the spore; but in the majority of cases the aplanospores arose singly within the cells of the filament, producing new cell-walls of their own, and were set free by the splitting of the mother-cell wall (Text-fig. 5, *e*) which was afterwards observed gradually to disintegrate.

#### IV. DISCUSSION OF THE POSSIBLE SIGNIFICANCE OF ALGAE AND MOSS PROTONEMA IN THE ECONOMY OF THE SOIL.

The results of the foregoing experiments, taken in conjunction with those of Esmarch, Robbins, and Petersen, indicate that there is in many soils a definite algal flora that is especially well developed in cultivated soils, even in those that would appear at first sight to offer no conditions suitable to the growth of algae, and that this algal flora may include blue-green algae, diatoms, and green algae. Esmarch observed that the blue-green algae found by him are not confined to the surface-layers of soil, but are to be found, more or less completely, in soils that have been cultivated, even to a depth of 10–25 cm., while a smaller number of species may be found as much as 40–50 cm. below the surface; and he observed that the species in the lower layers are almost identical with those of the surface-layers of the soil. He attributes this extensive distribution of blue-green algae in cultivated soils chiefly to the cultivation of the ground, in that by ploughing, digging, and hoeing not only are the conditions of the soil made more favourable to the growth of algae, but also fresh layers of soil are constantly being brought to the surface, and the surface-algae buried more and more deeply. He considers that the spores contained in the new surface-layers germinate on being exposed to the light and form fresh vegetative filaments on the surface, which at a later date are again buried. As other factors working for the distribution of algae in the soil he considers that the percolation of water and the burrowing of worms and other small animals are also effective.

The physiological experiments of Boresch,<sup>1</sup> Schindler,<sup>2</sup> and <sup>3</sup> and Magnus,<sup>2</sup> showing that certain algae are able to build up chlorophyll and phycocyanin and to grow in the dark, provided that sufficient nitrogenous food material is available, appear to have an important bearing on the mode of life of soil-algae, and suggest that it is quite possible for algae not merely to exist passively in the lower layers of the soil, but also to carry on active growth, for some time at any rate. In order to test this possibility Esmarch carried out a series of experiments upon algae specially buried in the soil at a known date and available for microscopic examination at any time; the cultures of the algae were kept in the dark.

From these experiments Esmarch observed that the blue-green algae enclosed in the soil retained their normal colour for some time, but that after a shorter or longer period, depending on the composition of the soil and the species of alga, the filaments gradually lost their blue-green colour and finally became yellowish. At first the dimensions and appearance of the filaments remained unchanged and the cells appeared perfectly healthy, but after some time distortion and finally disintegration of the filaments took place, leaving only spores and heterocysts.

He observed that *Nostoc* sp. had greater powers of resistance than *Anabaena* and *Cylindrospermum* spp., and attributed this to the fact that the mucilaginous investments and cell-walls are much stronger and more permanent in the *Nostoc* species than in the others.

Esmarch's work is thus extremely important in that it shows that not only is there an extensive flora of blue-green algae in the top layers of the soil, but also that filaments of at least some of these algae are able to continue their vegetative functions below the surface of the ground for periods varying in individual cases from three to six weeks, or even, as in the case of one *Nostoc* sp., as much as ten weeks.

Estimations of the approximate numbers of algae present in different soils in relation to other organisms have not yet been attempted, but the large number of different species found in English soils rather suggests that the proportions would be high for this country. No recent sample of a cultivated soil has been investigated which yielded fewer than four different species, and only four samples yielded fewer than seven species; many of the cultures were found to contain at least a dozen, while several yielded as many as seventeen or eighteen different species and varieties, in addition to the moss protonema which was obtained from every soil. It is inconceivable that such an extensive population of chlorophyll-containing organisms can be without its effect both on the other organisms in the soil

<sup>1</sup> Boresch: Die Färbung von Cyanophyceen und Chlorophyceen in ihrer Abhängigkeit vom Stickstoffgehalt des Substrats. Jahrb. für Bot., lii, 1913, pp. 145-85.

<sup>2</sup> Magnus, W., and Schindler, B.: Ueber den Einfluss der Nährsalze auf die Färbung der Oscillarien. Ber. der Deutsch. Bot. Ges., xxx, 1912-13, p. 314.

<sup>3</sup> Schindler, B.: Ueber den Farbenwechsel der Oscillarien. Zeitsch. f. Bot., v, 1913, pp. 553-5.

and on the soil itself, but up to the present too little is known on the subject to make any very definite statements in this direction.

It was thought by Frank<sup>1</sup> and by Schloesing and Laurent<sup>2</sup> that algae in the soil had the power of fixing atmospheric nitrogen, but later experiments by Kossowitsch<sup>3</sup> showed conclusively that algae by themselves are quite unable to carry on these activities. Kossowitsch suggested, on the contrary, that nitrogen fixation was the result of bacterial activity, and definitely proved this to be true in a few of his cultures. He observed, however, that the presence of certain algae in the soil is highly advantageous to nitrogen fixation, and concluded that there exists a symbiotic relationship between the algae and bacteria of the soil as a result of which nitrogen compounds are added to the soil. From the observation of Berthelot<sup>4</sup> that an increase in the nitrogen content of the soil does not continue unless fresh organic material is added to the soil, and that of Gautier and Drouin<sup>5</sup> that organic compounds destitute of nitrogen serve to stimulate nitrogen fixation in the soil, together with his own observation that the addition of sugar to certain cultures containing algae and bacteria made little difference to the amount of nitrogen fixed by the organisms, though in the absence of algae the difference was very considerable, Kossowitsch concluded that the bacteria were able to use certain organic compounds supplied by the algae. He suggested that the mucous sheaths always observed to be present round those algae having the greatest effect on nitrogen fixation probably provide the carbohydrate needed by the bacteria for growth; in return the algae are supplied with the nitrogenous substances without which they are unable to develop.

Further experiments by Bouilhac and Giustiniani<sup>6</sup> showed that in sand completely destitute of organic matter and of nitrogen compounds a mixture of soil-bacteria and algae are not only able to develop normally, but also to enrich the soil with nitrogen sufficiently to support the growth of

<sup>1</sup> Frank, B.: (a) Ueber den experimentellen Nachweis der Assimilation freien Stickstoffs durch erdbodenbewohnende Algen. *Ber. der D. Bot. Ges.*, vii, 1889, pp. 34-42.

(b) Ueber den gegenwärtigen Stand unserer Kenntnisse der Assimilation elementaren Stickstoffs durch die Pflanze. *Ber. der D. Bot. Ges.*, vii, 1889, pp. 234-47.

(c) Frank, B., and Otto, R.: Untersuchungen über Stickstoff-Assimilation in der Pflanze. *Ber. der D. Bot. Ges.*, viii, 1890, pp. 331-42.

<sup>2</sup> Schloesing, fils, and Laurent, E.: Recherches sur la fixation de l'azote libre par les plantes. *Ann. de l'Institut Pasteur*, vi, 1892, pp. 65-115.

<sup>3</sup> Kossowitsch, P.: Untersuchungen über die Frage, ob die Algen freien Stickstoff fixiren. *Bot. Zeit.*, 1894, Heft 5, pp. 98-116.

<sup>4</sup> Berthelot, M.: Recherches nouvelles sur les microorganismes fixateurs de l'azote. *Compt. Rend.*, cxvi, 1893, pp. 842-9.

<sup>5</sup> Gautier and Drouin: Recherches sur la fixation de l'azote par le sol et les végétaux. *Compt. Rend.*, cvi, 1888, pp. 754, 863, 944, 1098, 1174, 1232, 1605.

<sup>6</sup> (a) Bouilhac, R.: Sur la fixation de l'azote atmosphérique par l'association des algues et des bactéries. *Compt. Rend.*, cxxiii, 1896, pp. 828-30.

(b) Bouilhac, R., and Giustiniani: Sur une culture de sarrasin en présence d'un mélange d'algues et de bactéries. *Compt. Rend.*, cxxxvii, 1903, pp. 1274-6.



higher plants; while still more recently Pringsheim<sup>1</sup> has shown that the ability of bacteria to fix nitrogen is closely dependent upon the presence of blue-green algae.

Esmarch<sup>2</sup> and Robbins<sup>3</sup> both incline to the belief that in certain soils at any rate the presence of algae, especially of blue-green algae, is an important factor in the fixation of nitrogen by bacteria. In certain of the Colorado soils large quantities of nitrate are built up through the agency of *Azotobacter chroococcum* despite the fact that these soils are poor in organic matter, and Robbins considers that the bacteria probably derive their organic material from the mucilaginous investments of the blue-green algae, which he shows to be present in considerable numbers.

Recently Nakano<sup>4</sup> investigated the relationship existing between algae and *Azotobacter* and shows that it holds not only for blue-green algae but also for certain green algae. He points out that the pure cultures of *Azotobacter* fix less nitrogen than similar cultures to which pure algae have been added, and also shows that these green algae are able to build up chlorophyll in the dark not only on organic culture-media but also in aqueous culture-solutions of mineral salts into which sugar has been introduced. Nakano did not deal specifically with soil-forms, but both of these observations have a good deal of significance in considering the possible economic functions of soil-algae.

According to Russell<sup>5</sup> the activities of *Azotobacter* are limited to well-aerated soils that are sufficiently provided with calcium carbonate, potassium salts and phosphates, carbonaceous material of the right kind, and moisture, and do not take place except at a comparatively high temperature. He admits, however, that is impossible to argue from a culture-solution to a soil, and Robbins's statement that *Azotobacter chroococcum* is the chief nitrogen-fixing bacterium in Colorado soils that are poor in organic material rather suggests that *Azotobacter* may work more efficiently in conjunction with blue-green algae than with the sugar and other carbohydrates used in the cultures of Koch, Pringsheim, and other investigators.

In their researches on the fixation of nitrogen by the soil Gautier and Drouin<sup>6</sup> observed that in unsown soils which contain only ammonia as their source of nitrogen and are destitute of organic matter, there is a constant decrease in the amount of ammonia present which is due partly to its slow conversion into organic substances in the soil and partly to its escape into the air. But if algae are present the amount of nitrogenous organic

<sup>1</sup> Pringsheim, E.: Kulturversuche mit chlorophyllführenden Mikroorganismen, III: Zur Physiologie der Schizophyceen. Cohns Beiträge z. Biol. d. Pflanzen, Bd. xii, pp. 99-107.

<sup>2</sup> Esmarch: loc. cit., 1914.

<sup>3</sup> Loc. cit.

<sup>4</sup> Nakano, H.: Untersuchungen über die Entwicklungs- und Ernährungsphysiologie einiger Chlorophyceen. Journ. of Coll. of Science, Imperial Univ., Tokyo, vol. xl, 1917, Art. 2, p. 66 &c.

<sup>5</sup> Russell, E. J.: Soil Conditions and Plant Growth. Monographs in Biochem., 1915, p. 97.

<sup>6</sup> Gautier and Drouin: loc. cit., pp. 1174-6, 1232-4.

substance built up is greatly increased, while the loss of ammonia into the air is reduced to a minimum ; similar results were also obtained with soils in which higher plants were growing. Hence Gautier and Drouin regarded soil-algae less as factors in the acquisition of nitrogen by the soil than as agents for the transformation of the ammoniacal substances already present into more complex organic substances, and thus as factors in helping to bring about the nitrogen cycle of the soil.

This consideration of the facts already ascertained indicates very clearly that the part that living algae play in the economy of the soil is no inconsiderable one, though the difficulties in the way of establishing direct evidence are very great, and much work will be necessary before any very definite statements can be made. It is, on the other hand, quite certain that by their death algae contribute very largely to the fertility of soils in that they present considerable quantities of organic material to the putrefactive bacteria for decomposition.

The extraordinary property which moss protonema and many of these soil-algae possess, of being able to retain their vitality for very long periods, even under conditions of complete drought, has already been described elsewhere;<sup>1</sup> and it is to be emphasized in this connexion that all of the forms which exhibit these powers build up considerable quantities of a fatty oil in their cytoplasm, so that the chemical energy stored up in them is very high, and their value as sources of energy for other organisms correspondingly great. In the cultures under observation certain protozoa have been observed to feed quite extensively on soil-algae, specimens being frequently found containing a dozen or more inclusions that might be either diatoms or green algae, or more rarely blue-green algae. Whether this condition exists in nature or is merely an accident due to the presence of a greater amount of water than is usually available under natural conditions in the soil it is impossible to say, but it is probable that algae may also contribute largely to the nutrition of some of the lower organisms of the soil, and especially of worms.

Again, if algae are present in any quantity in a soil, their physiological functions of respiration and photosynthesis must have a certain effect upon the nature of the gases in the soil, while their mucous investments probably play an important part, especially in sandy soils, in helping the soil to retain its moisture.

<sup>1</sup> Bristol, B. M. : (a) On the Remarkable Retention of Vitality of Moss Protonema. *New Phyt.*, vol. xv, No. 7, July, 1916, p. 137.

(b) On the Retention of Vitality by Algae from Old Stored Soils. *New Phyt.*, vol. xviii, Nos. 3 and 4, 1919.

V. DESCRIPTIVE NOTES ON THE SPECIES FOUND.

**Myxophyceae.**

1. *Phormidium tenue*, (Menegh.) Gomont.

This species, found in twenty different samples of soil, is the commonest blue-green alga in the soils examined. As observed in the cultures, it agreed exactly in dimensions and all other characters with the typical form from which the species was described.

2. *Phormidium Bolneri*, Schmidle.

Up to the present time this species has been described only from damp soil in Africa, but there is no doubt that a *Phormidium* found growing from four of these soil-samples must belong to this species. The algal filaments were almost straight, but slightly curved and interwoven to form a thin pale blue-green stratum. The cells were usually a little shorter than broad, but might be of equal dimensions or rather longer than broad; no constrictions were observed between the cells, and the end cell was generally rounded, though occasionally it was somewhat swollen and formed a knob-like termination to the filament. The sheath was colourless and rather thin, and the breadth of the filament about  $2\ \mu$ .

3. *Phormidium laminosum*, (Ag.) Gomont.

The alga identified under this name occurred in one soil only, but the extraordinarily varied habitat already described for this species makes the record an extremely interesting one; there are few species of algae capable of living in places so widely different as hot springs, standing water, rocks moistened by spray from a waterfall, and garden-soil. The form observed in the cultures agreed almost exactly with the typical form, but the filaments were slightly wider, being  $2\ \mu$  in breadth instead of only  $1.5\ \mu$ .

4. *Phormidium autumnale*, (Ag.) Gomont.

Next to *P. tenue*, this species occurred most frequently in the soils examined, having been found in the cultures of nineteen samples. The filaments were interwoven to form expanded strata of a deep brownish-green or almost black colour on the surface of the soil and on the sides of the culture-vessel. The ends of the filaments were slightly tapering; the end cell was sometimes capitate and sometimes rounded, and in this case the last two or three cells of the filament might be slightly curved. The breadth of the filaments was  $4-8\ \mu$ .

5. *Lyngbya Kuetsingii*, Schmidle.

A blue-green alga very closely resembling this species was found in the cultures of seven of the soils, with usually straight but occasionally curved filaments matted together to form a dark brownish-green expanded stratum. The filaments were  $3$  to  $3.5\ \mu$  broad, bounded by a strong colourless sheath; and the end cells were rounded without being tapering. Very

occasionally a slight constriction between the cells could be observed, but this was not general. This species has previously been recorded only from running water, where it grows attached to water-plants, hence its appearance from the soil-samples is surprising.

6. *Nostoc muscorum*, Kuetz.

This species was obtained from five soils, but, possibly owing to cultural conditions, it differed slightly from the typical form. Young colonies were globular, with closely matted filaments coiled in loops towards the periphery of the colony, where the sheaths of the individual filaments could be distinguished. The whole was surrounded by a firm mucous sheath that gradually became light brown in colour. The colonies retained this form until they were 100 to 200  $\mu$  in diameter, but sooner or later they split to form an expanded leaf-like stratum in which the characters of the filaments could be distinguished. The cells were spherical or shortly barrel-shaped, 3 to 5  $\mu$  broad; the heterocysts almost spherical and 5 to 7  $\mu$  broad; the spores 6 to 8  $\mu$  long by 8 to 9.5  $\mu$  broad, arranged in long chains and possessing smooth yellowish walls.

7. *Nostoc foliaceum*, Mougeot.

A blue-green alga that could definitely be identified as this species was obtained from only two soils, but a form very closely resembling it was obtained from two others and may be merely a cultural form of the same species. In the first of these two forms the young colonies were spherical or irregularly elongated, consisting of filaments densely woven together and surrounded by a definite mucous sheath which gradually became yellowish-brown in colour. Later the colonies expanded to form flat and somewhat diffuse strata of more loosely entwined filaments. The vegetative cells were spherical or shortly barrel-shaped and 4 to 4.5  $\mu$  broad; but heterocysts were never formed in the cultures, and the spores were rather smaller than is typical for the species, being only 5.5 to 7  $\mu$  broad by 6.5 to 10  $\mu$  long.

In the second form the general features of the colonies were very similar to those of the first, but the vegetative cells were shortly barrel-shaped and 5 to 6  $\mu$  broad, while such few heterocysts as were formed in the cultures were exceedingly small, being only 4 to 5  $\mu$  in diameter. This reduction in size and frequency of the heterocysts appears, however, to be a fairly common character in cultural forms, and therefore too much importance cannot be attached to it. The spores occurred in large masses and were oval or subspherical, about 6 to 9  $\mu$  in diameter. This is the first time that the species has been recorded from Great Britain.

8. *Nostoc commune*, Vaucher (Text-fig. 4).

Forms of this species were found in three arable soils. In the first form (A) the vegetative cells were barrel-shaped, 4 to 6  $\mu$  in diameter, and the heterocysts subspherical and about 6.5  $\mu$  in diameter. Spores were

formed in long chains; they were oval,  $4.5$  to  $7\ \mu$  broad by  $6$  to  $7.5\ \mu$  long, with smooth colourless walls.

The second form (B), though by its mode of growth and general characters obviously belonging to the same species, differed a little in its dimensions. The vegetative cells were mostly nearly spherical,  $4.5$  to  $5.5\ \mu$  in diameter; the heterocysts were somewhat reduced in size, being only  $4.5$  to  $6\ \mu$  in diameter, and two or sometimes three were frequently found in series. The spores were oval, and might have their long axis placed either longitudinally or transversely; the walls were smooth and colourless, the breadth  $7$  to  $8\ \mu$ , and the length  $10$  to  $11\ \mu$ . It is impossible to say from the evidence obtained which form of spore is to be regarded as more typical of the species; form (A) was observed in the cultures of two samples, while form (B) was found only in one, but it is quite possible that (B) represents a later stage of development than (A), though, on the other hand, the small size of the heterocysts in (B) rather indicates that it may be a somewhat abnormal form due to cultural conditions.

9. *Nostoc sphaeroides*, Kuetz.

A blue-green alga appeared in two of the cultures which agreed more nearly with this species than with any other, and was probably only a cultural form of it. The young colonies were globular, consisting of filaments very closely entwined together and with a close gelatinous envelope. The vegetative cells were shortly barrel-shaped, or nearly spherical and  $5$  to  $7\ \mu$  in diameter. The ends of the filaments were somewhat attenuated, and the terminal cells longer in proportion to their diameter. Heterocysts were not produced in the cultures, but spores were formed in long chains; they were subspherical or spherical,  $10$  to  $13\ \mu$  in diameter, and frequently embedded in a wide and somewhat diffuse mucous sheath. At maturity the spore-wall acquired a golden-brown colour, but was never observed to become rough, as has been described for this species. It is possible, however, that this variation may be the effect of cultural conditions, since it has been observed that in the alga *Trochiscia aspera*, (Reinsch) Hansg., there is a great tendency in cultures for the cell-wall to lose its characteristic external ornamentation. This species has not previously been recorded from the British Islands.

10. *Nostoc minutum*, Desmaz. (Text-fig. 6).

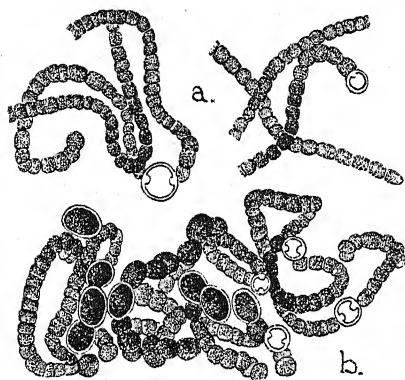
This interesting little species was found in the cultures of only one sample of soil, but its presence there is all the more noteworthy since it was observed, for the first time on record, to produce spores. The colonies were extremely small and rather irregular in shape, consisting of closely coiled filaments embedded in a definite colourless mucous envelope. The vegetative cells were barrel-shaped, about  $2.5\ \mu$  broad, and the end cell of the filament was conical. The heterocysts were subspherical, and somewhat variable in size; the majority were  $3.5$  to  $4.5\ \mu$  in diameter, but

occasional heterocysts were found whose diameter was as much as  $6\mu$ . The spores were formed in series and were oval with smooth pale-yellow walls; they were  $4.5$  to  $5\mu$  broad and  $6$  to  $7\mu$  long.

11. *Nodularia Harveyana*, (Thwaites) Thuret.

The form of this alga, which appeared in four of the samples, very closely resembled that already described from the old stored soils from the Rothamsted Experimental Station,<sup>1</sup> but was somewhat larger.

The vegetative cells were barrel-shaped, about  $4$  to  $6\mu$  broad and about half as long. Heterocysts were completely absent, and the spores were sub-spherical and about  $8\mu$  in diameter, or oval and about  $9\mu$  broad by  $11$  to  $15\mu$  long, usually about  $13\mu$ . There was frequently a distinct mucous sheath surrounding the filament.



TEXT-FIG. 6. *Nostoc minutum*, Desmaz.  
a., vegetative filaments; b., spore-formation.  
 $\times 825$ .

12. *Anabaena variabilis*, Kuetz.

This species occurred only in one soil-sample, and is recorded here for the first time from the British Islands. The vegetative cells were barrel-shaped,  $4.5$  to  $6\mu$  broad, and usually shorter than broad, with slight constrictions between the cells. The heterocysts were spherical or subspherical and  $6.5$  to  $7\mu$  in diameter. The spores were  $7$  to  $9.5\mu$  broad and  $8$  to  $15\mu$  long, with smooth yellowish-brown walls; they grew in series, but were to be found sometimes separated

from and sometimes adjacent to the heterocysts, so that the form in the cultures was not exactly typical in this respect.

13. *Anabaena inaequalis*, (Kuetz.) Born. et Flah., forma.

A somewhat large form of this alga was found in five of the soils. The filaments were almost straight and parallel and were usually loosely entwined to form an extensive stratum. The vegetative cells were shortly barrel-shaped,  $5$  to  $6\mu$  broad, and about  $4\mu$  long, with slight constrictions between the cells. The filaments gradually tapered towards the end, and the end cell was rounded. The heterocysts were spherical and  $6.5$  to  $8\mu$  in diameter; and the spores were cylindrical,  $8$  to  $9\mu$  broad, and  $14$  to  $18\mu$  long, with smooth yellowish walls. The spores were formed singly or in series of two, three, or as many as six, either adjacent to or apart from the heterocysts. This alga, though differing in dimensions from the typical form, agrees so much more nearly with it than with any other described species that it can only be regarded as a form of this species.

<sup>1</sup> Bristol, B. M.: loc. cit., 1919.

14. *Anabaena sphaerica*, Born. et Flah.

In the cultures of two of the soils a blue-green alga appeared which resembled this species more nearly than any other and is probably a form of it, somewhat larger than is typical.

The vegetative cells were 5 to 6  $\mu$  or even as much as 8  $\mu$  broad, and shortly barrel-shaped. The heterocysts were spherical, 7 to 8.5  $\mu$  broad or smaller in young filaments. The spores grew in series on one side of the heterocysts, and had a smooth yellowish-brown wall; they were oval and about 10 to 12  $\mu$  broad by 11 to 19  $\mu$  long when ripe. This species has not previously been recorded from the British Islands.

15. *Anabaena oscillarioides*, Born., var. *terrestris*, Bristol, forma *minor*.

This alga occurred in five of the soil samples, but it has already been fully described and figured elsewhere,<sup>1</sup> hence no further particulars need be given here.

16. *Anabaena* sp.?

In the cultures of three of the soils an *Anabaena* was found which, owing to its imperfect development, could not be identified with any of the described species. The vegetative cells were barrel-shaped, 5 to 6  $\mu$  in diameter and usually about 4  $\mu$  long, though they might be rather longer than broad, and a thin mucous sheath could sometimes be seen surrounding the filament, the terminal cell of which was conical.

Heterocysts were numerous throughout the length of the filament, and were yellowish in colour and subspherical, about 8  $\mu$  in diameter, or even occasionally up to 9.5  $\mu$ . The spores were formed apart from the heterocysts, either singly or in short series of two or three, and when fully ripe appeared to be oval-cylindrical and about 11 to 12  $\mu$  broad by about 27  $\mu$  long, with a smooth brownish spore-wall. There was, however, so much variation in the size of the spores that it was impossible to decide which species the form most closely resembled.

17. *Cylindrospermum majus*, Kuetz.

This alga was found in two soil-samples, and except for the somewhat small size of the spores was quite typical of the species. The vegetative cells were 3.5 to 4  $\mu$  broad and 4 to 5  $\mu$  long; the heterocysts elongated, about 5.5 to 6  $\mu$  broad and 6.5 to 10  $\mu$  long; and the spores solitary, with a papillose, dark greenish-brown wall, about 11 to 12  $\mu$  broad and 18 to 22  $\mu$  long.

18. *Cylindrospermum licheniforme*, (Bory) Kuetz.

This species was found in the cultures of four soils. Vegetative filaments were only rarely seen, and when found the cells appeared to be somewhat large for the species, about 4.5 to 5  $\mu$  in diameter and a little longer than broad. The very characteristic spores, 11 to 14  $\mu$  broad and 24 to 36  $\mu$  long, with their dark red-brown walls and truncate apices, left no

<sup>1</sup> Bristol, B. M.: loc. cit., 1919.



doubt, however, as to the identity of the alga. The heterocysts were usually oval, 5 to  $6\mu$  broad by  $7.5$  to  $8.5\mu$  long, but occasionally they were subspherical, about 6 to  $7.5\mu$  in diameter.

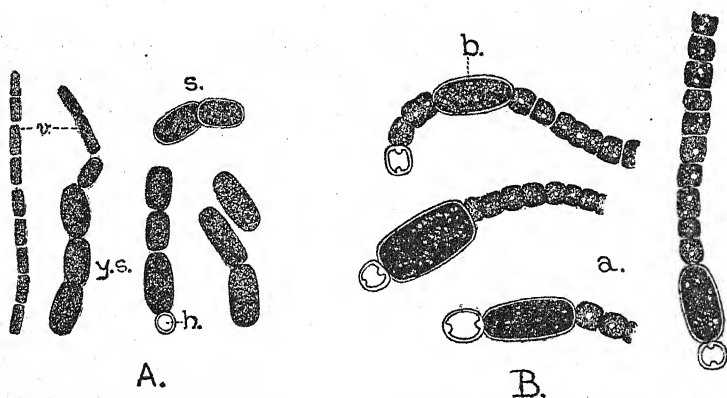
19. *Cylindrospermum muscicola*, Kuetz. (Text-fig. 7 B).

In two of the soils a blue-green alga was found which agreed very nearly with this species, though it differed in certain respects. The vegetative cells were  $3.5$  to  $4\mu$  broad, but were either equal in length or somewhat shorter than broad; one or two conspicuous clear granules were observable in each cell. The heterocysts were oval and mostly 4 to  $5\mu$  broad and 5 to  $8.5\mu$  long, though they might be longer, while the spores were cylindrical-oval, 9 to  $10.5\mu$  broad and  $17.5$  to  $24\mu$  long, with smooth golden-brown walls.

At *b* in Text-fig. 7, B, is shown an abnormal filament in which the spore had begun to develop at a little distance from the heterocyst instead of adjacent to it. The species is here recorded for the first time from Great Britain.

20. *Cylindrospermum marchicum*, Lemm., forma *tenuis*, n.f.

In Text-fig. 7, A, is figured a blue-green alga very closely resembling *C. marchicum* in all its general characters, but differing from it considerably in point of size; this form was observed in the cultures of only one soil. The vegetative cells were cylindrical,  $1.5$  to  $2\mu$  broad and  $3.5$  to  $6\mu$  long,



TEXT-FIG. 7. A. *Cylindrospermum marchicum*, Lemm., forma *tenuis*, n.f. h., heterocyst; s., ripe spores; y.s., young spores in series; v., vegetative cells. B. *Cylindrospermum muscicola*, Kuetz. a., typical filaments in different stages of spore-formation; b., spore formed in an irregular position.  $\times 825$ .

with conspicuous constrictions between the cells; the apical cell of the filament was conical. Only very few heterocysts were observed, but they were subspherical, about  $2.5\mu$  broad and  $3\mu$  long. The spores were cylindrical, with smooth colourless walls, and 4 to  $4.5\mu$  broad by 9 to  $11\mu$  long; they grew in series of three or four together. The extremely elongated vegetative cells and the seriate spores with colourless walls

make it very probable that this alga is only a small form of *C. marchicum*, Lemm., a species not previously known to occur in this country.

21. *Plectonema Battersii*, Gomont.

The soil-form of this alga, which was observed in the cultures of no less than thirteen soil-samples, produced flat expanded strata of a dark brownish-green or black colour on the sides of the culture-vessels, consisting of numerous long filaments entwined together and repeatedly branched. The false branches were produced singly or in pairs, and were very slightly narrower than the main filaments and somewhat tapering towards the end; the filaments were 2.5 to 3  $\mu$  broad and the cells considerably shorter than broad, with slight constrictions between them; the end cell was rounded. The cell-contents were homogeneous and pale blue-green.

22. *Scytonema javanicum*, (Kuetz.) Bornet.

The alga which has been identified under this name was found in two soils only, but in a third soil germinating spores of a similar form were found which never completely developed, and it is probable that they were early stages of growth of the same species. The filaments were agglutinated together and the sheaths were thin, becoming deep yellow; they were usually about 12  $\mu$  in breadth. The vegetative cells were usually 9 to 10  $\mu$  broad and from 5 to 8  $\mu$  long. The heterocysts were brownish and hemispherical at the bases of the filaments, or compressed interstitially, but there was every indication in the cultures that the alga in question had not reached its mature form, and it is possible that at a later stage of development the heterocysts would obtain a more constant shape. The false branches were observed to grow singly and in pairs. This species has not previously been recorded from the British Islands.

**Bacillarieae.**

1. *Navicula borealis*, Ehr. (Text-fig. 8, 1 and 2).

This species was observed in nine soils, and was one of the largest diatoms growing in the cultures. The form found was exactly typical of the species, but somewhat small, being only 40 to 44  $\mu$  long by 8.5  $\mu$  broad. There were 6 costae in 10  $\mu$ . The raphe was bent in the same direction on both sides of the median nodule and at the terminal nodules.

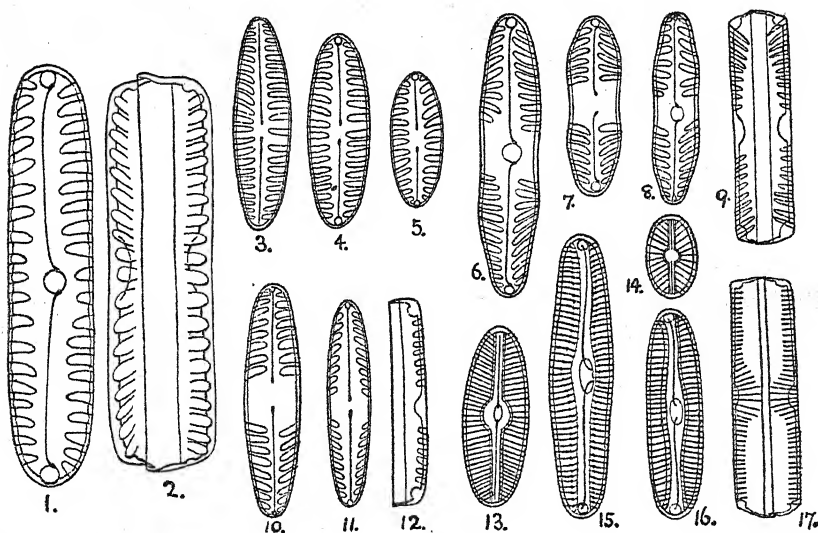
2. *Navicula Balfouriana*, Grun. (Text-fig. 8, 3-5).

The diatom that has been identified as this species agreed almost exactly with that figured under the same name in Schmidt's 'Atlas der Diatomaceen-Kunde', Heft 79, Tafel 313, Figs. 29-31. The costae were continuous along the whole length of the valve, and converged slightly towards the middle; there were usually 10 costae in 10  $\mu$ , rarely 11 or 12, and the valve was oval, 18 to 24  $\mu$  in length by 5 to 6  $\mu$  in breadth. The raphe was not bent in the same direction on both sides of the median

nodule, however, but appeared to be quite straight. This form occurred in thirteen soil-samples.

3. *Navicula Brebissonii*, Kuetz., var. *diminuta* (Text-fig. 8, 10-12).

This diatom was also found in thirteen soil-samples. The valves were narrowly elliptical, about 19 to 24  $\mu$  long and 4.5 to 5  $\mu$  broad, with 10 to 11 costae in 10  $\mu$ . The costae were mainly divergent and did not reach to the raphe; they were interrupted opposite the median nodule by a wide space; those nearer the middle were usually shorter than the rest. The raphe appeared to be quite straight. This form differed from the one originally described in that the central costae were not converging as a rule, but the agreement was so close in all other respects that it seems best to include it in the same variety.



TEXT-FIG. 8. Soil-diatoms I. 1 and 2, *Navicula borealis*, Ehr.; 3-5, *N. Balfouriana*, Grun.; 6-9, *N. intermedia*, Lagerst.; 10-12, *N. Brebissonii*, Kuetz., var. *diminuta*, Van H.; 13, *N. elliptica*, Kuetz., var. *oblongella*, Naeg.; 14, *N. elliptica*, var. *minima*, Van H.; 15-17, *N. terricola*, n. sp. Figs. 1-14,  $\times 1435$ ; figs. 15-17,  $\times 1715$ .

4. *Navicula intermedia*, Lagerst. (Text-fig. 8, 6-9).

Petersen has figured a diatom which he considers to be a form of this species (loc. cit., p. 293, Fig. 19) obtained from three Danish soils. A diatom almost exactly resembling Petersen's form has appeared in the cultures of four soil-samples in the present work, and evidently belongs to the same species, though no previous record has been made of its existence in this country. The valves are slightly constricted in the middle and have somewhat capitate apices; they vary in length from 17 to 27  $\mu$ , and in

breadth from  $4.5$  to  $6\mu$ . The costae are convergent towards the middle and divergent towards the apices, and are interrupted by a wide space opposite the central nodule; there are about 10 to 11 striations in  $10\mu$ . The raphe is bent in the same direction on both sides of the central nodule and at the terminal nodules.

5. *Navicula elliptica*, Kuetz.

(a) var. *oblongella*, Naeg. (Text-fig. 8, 13).

A form appeared in the cultures of one of the soils which agreed more nearly with this variety than with any other described form, and which certainly seemed to be very closely allied to it. The valves were oblong-elliptical, about  $17\mu$  long and  $6.5\mu$  broad, with an elongated hyaline area on both sides of the raphe swelling out into a conspicuous circular area around the median nodule, which was oval in shape.

The striations were gradually radiating from the central nodule, about 20 in  $10\mu$ , and were interrupted near the raphe by a longitudinal furrow which was exactly parallel to the edge of the hyaline area.

(b) var. *minima*, Van H. (Text-fig. 8, 14).

A small oval diatom, rarely more than  $8\mu$  long, with a hyaline area and longitudinal furrow exactly similar to that of the above form, was found in two soils. The striations were radiating and about 17 to 18 in  $10\mu$ .

6. *Navicula terricola*, n. sp. (Text-fig. 8, 15-17).

In one of the soils a diatom was found which resembled *N. elliptica*, var. *oblongella*, very closely, but which differed from it both in the more oblong form of the valve and in the character of the raphe. The length of the valve varied from  $16.5$  to  $22.5\mu$ , and the breadth from  $4.5$  to  $5\mu$ . The striations were slightly curved, almost perpendicular to the raphe near the median nodule and somewhat divergent towards the apices; there were about 21 to 25 in  $10\mu$ . The raphe was not straight, but the two halves were slightly sigmoid. The median nodule was oval and the terminal nodules circular. The raphe was surrounded by a conspicuous elongated hyaline area which was gradually dilated around the median nodule and towards the apices. The striations appeared to be interrupted near the raphe by a longitudinal furrow parallel to the edge of the hyaline area. No species has previously been described of which this diatom might be a form, hence it seems most satisfactory to regard it as a new species, nearly related to *N. elliptica*. The following is a diagnosis of the species: Valva lineari-elliptica, apicibus rotundis, media parte interdum nonnihil inflata. Raphae citra utraque medium nodulum leviter sigmoidea, zona circumdata conspicua elongata hyalina circa nodulum atque apices versus dilatata. Striae leviter flexae, raphae media parte fere perpendiculares apices versus nonnihil divergentes, sulcis duobus ad zonae hyalinae margines parallele interruptae.

Valvae  $16.5$  to  $22.5\mu$  longae,  $4.5$  to  $5\mu$  latae, striae 21 to 25 in  $10\mu$ .

*Hab.* in solo culto Harborne (Birmingham).

7. *Navicula* (§ *Diploneis*) *hyalina*, Donk., var. *minima*, n. var. (Text-fig. 9, 18–21).

An extremely minute diatom, which appeared, so far as it was possible to make out the details of its structure, to be a reduced form of *N. hyalina*, Donk., was found in two of the cultures. The valves were extremely delicate, about 11 to 13.5  $\mu$  long by 3.5 to 4.5  $\mu$  broad, and so hyaline that it was extremely difficult to make out the details. The striations were extremely faint and could only be distinguished rarely and with difficulty; they appeared to vary from about 27 to 37 in 10  $\mu$ . The raphe lay in a furrow which in Text-fig. 9, 18 and 19, is shown to terminate abruptly near the median nodule, and in Text-fig. 9, 21, to be contracted at the apices. In certain individuals (Text-fig. 9, 18 and 19) there appeared to be a double median nodule, but the frustules were so small that it is probable that this was merely a reduced form of the structure figured for *N. hyalina*, Donk., in Schmidt's 'Atlas', Pl. 70, Figs. 4 and 5; the terminal nodules were conspicuous. In all of the frustules there was visible a longitudinal furrow (?) running parallel to the margin of the valve about half-way between the margin and the raphe.

It is quite possible that this minute form may be a new species, but the general resemblance in structure to *N. hyalina*, Donk., makes it more satisfactory to regard it as simply a minute variety of this species.

8. *Navicula mutica*, Kuetz. (Text-fig. 9, 1–7).

This extremely variable diatom was observed in the cultures of seventeen different soil-samples, and appears to be one of the commonest soil-diatoms. A number of different forms were found, of which one (Text-fig. 9, 5) appeared to be identical with that described by Grunow as *N. Kotschy*; this distinction seems, however, to be quite unnecessary since the characters of the diatom agreed extremely well with those of *N. mutica*. An interesting feature in this species was the great variation in the size of the puncta which made up the transverse striations. As shown in the figures, they were sometimes exceedingly small (Text-fig. 9, 1 and 7), but they might be much larger so that only five or six were found in each striation (Text-fig. 9, 2–4). Sometimes the puncta appeared to be arranged in no definite order, but in other individuals they were arranged in more or less regular longitudinal rows on the valve (Text-fig. 9, 3). In the great majority of frustules there were 18 to 19 striations in 10  $\mu$ , but in Text-fig. 9, 2 and 6, are shown individuals with only 14 to 16 striations in 10  $\mu$ , the one in Text-fig. 9, 6, being still farther removed from the typical form by the oval shape of the valve.

The breadth of the valve varied from 5 to 7.5  $\mu$  and the length from about 10  $\mu$  to nearly 30  $\mu$ .

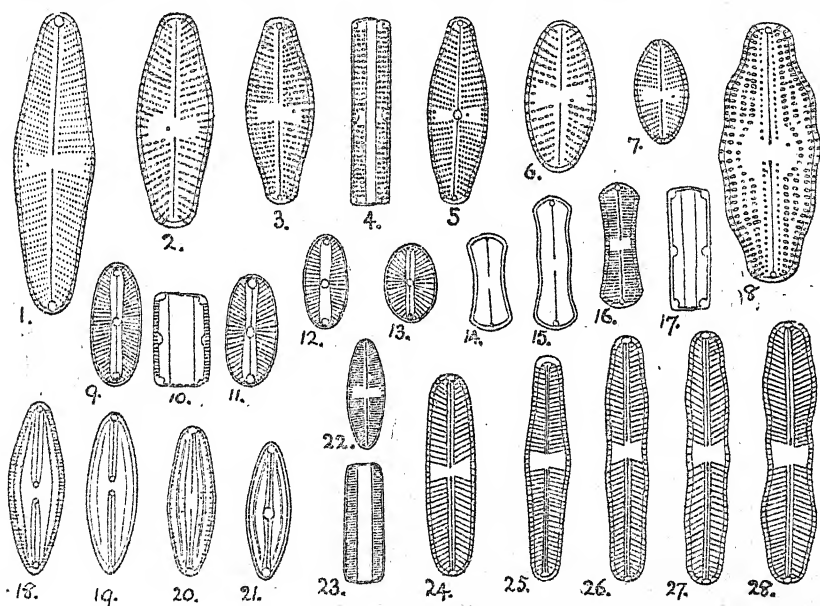
var. *quinquenodis*, Del. (Text-fig. 9, 8).

This very characteristic variety of the species was found in four soils, and was observed to have the puncta arranged in very definite longitudinal rows so as to produce a regular pattern on the surface of the valve, as shown

in the figure. The puncta were of variable size according to their position, the one terminating each striation near the raphe being very small and circular, while most of the others were larger and frequently oval in shape. The valve was  $10\mu$  broad and about  $25\mu$  long, with 17 striations in  $10\mu$ .

9. *Navicula Pupula*, Kuetz. (Text-fig. 9, 24 and 25).

This diatom, as observed in the cultures of eleven soils, showed a considerable tendency to variation in shape. The valves were elongated and usually swollen opposite the median nodule, with rounded apices (Text-fig. 9, 25); but sometimes they were narrowly elliptical without any enlargement in the centre (Text-fig. 9, 24). There was a straight hyaline zone surrounding the raphe and swelling out abruptly opposite the median nodule to form a false stauros. The striations were radiating, and varied in number from



TEXT-FIG. 9. Soil diatoms II. 1-7, *Navicula mutica*, Kuetz. formae, 5 possibly *N. Kotschy*, Grun.; 8, *N. mutica*, var. *quinguenodis*, Del.; 9-13, *N. Atomus*, Naeg.; 14-17, *N. contenta*, Grun., var. *biceps*, Del.; 18-21, *N. hyalina*, Donk., var. *minima*, n. var. (transverse striations doubtful); 22 and 23, *N. exilissima*, Grun.; 24 and 25, *N. Pupula*, Kuetz. and formae; 26-8, *N. Pupula*, var. *undulata*, n. var. Figs. 1-17 and 22-8,  $\times 1435$ ; figs. 18-21,  $\times 1715$ .

20 to 24 in  $10\mu$ . The terminal nodules were enlarged sideways, but not to the extent that is figured in Cleve and Grunow's 'Die arctischen Diatomeen', Taf. II, Fig. 53; the valves were about 19 to 21  $\mu$  long and 4 to 4.5  $\mu$  broad, var. *undulata*, n. var. (Text-fig. 9, 26-28).

In two of the soils containing *N. Pupula*, Kuetz., a second form was observed in considerable quantity which differed from the latter in its somewhat larger size, 23 to 25  $\mu$  long by 4 to 5  $\mu$  broad, and in the shape of the valves. These were oblong with obtuse rounded ends and undulating

sides; the number of undulations varied in different individuals, being either two (Text-fig. 9, 26) or three (Text-fig. 9, 27 and 28). The striations were usually 22 to 24 in  $10\mu$ , rarely about 19. In all other respects this diatom agreed with the above species, but the general configuration of the valves was so characteristic that it seemed best to regard it as a special variety, under the name *undulata*.

10. *Navicula exilissima*, Grun. (Text-fig. 9, 22-23).

The markings on this minute diatom, found in two soils, were very difficult to see, but there were about 41 or 42 striations in  $10\mu$ . The valves were 10 to  $11\mu$  long by about  $3.5\mu$  broad, and elliptical in shape with somewhat pointed rounded ends. The striations were interrupted in the middle, producing a false stauros, and did not quite extend to the raphe. The species has not previously been recorded for this country.

11. *Navicula Atomus*, Naeg. (Text-fig. 9, 9-13).

The extremely characteristic form of the raphe in this species made its identification comparatively easy. It was found in sixteen different soil-samples, though this is the first record of its existence in the British Isles, and was oval in shape, usually 9 to  $11\mu$  long by  $4.5\mu$  broad, and extremely delicate. The raphe was surrounded by a clear hyaline zone which did not expand opposite the median nodule to form a false stauros; the striations, about 26 to 30, frequently 28, in  $10\mu$ , radiated out from the central nodule. This is a considerably larger form of the species than that described by Van Heurck.

12. *Navicula contenta*, Grun., var. *biceps*, Del. (Text-fig. 9, 14-17).

This species has not previously been recorded from the British Isles, yet fourteen different soil-samples yielded the variety *biceps*, usually in considerable quantity. The markings were difficult to make out, but the very characteristic form of the valves, which were united into long chains, left no doubt as to their identity. The valves were 9 to  $12\mu$  long and 3 to  $3.5\mu$  broad; while the swelling opposite the median nodule was either absent or feebly developed. There appeared to be about 38 striations in  $10\mu$ , with a gap in the middle producing a false stauros, but in the majority of cases it was impossible to see this. The siliceous wall was very thick in comparison with other diatoms of the same size.

13. *Hantzschia amphioxys*, (Ehr.) Grun. (Text-fig. 10, 1 and 2).

The forms of this ubiquitous species which occurred in the cultures were usually small, about 30 to  $40\mu$  in length by  $6.5$  to  $7\mu$  in breadth, but occasionally larger forms were observed, even up to  $60\mu$  in length. In the larger form (Text-fig. 10, 1) there were 7 carinal points in  $10\mu$  and about 16 to 17 striations; but in the smaller forms the carinal points were more numerous, frequently 8 to 9 and sometimes even 10 in  $10\mu$ , while there were about 20 striations in  $10\mu$ . In each case the two median carinal points were somewhat widely separated and a rudimentary nodule could be seen between them.



14. *Nitzschia obtusa*, W. Sm., var. *scalpelliiformis*, Grun. (Text-fig. 10, 7).

A small form of this diatom was found in one of the soils. The length of the valves was only about  $45\mu$ , but they were abruptly sigmoid and showed at the middle of their length a distinct inflexion. At this point there was a rudimentary nodule lying between the two median carinal points, which were somewhat separated from one another. There were 7.5 carinal points in  $10\mu$  and 26 striations.

15. *Nitzschia Palea*, (Kuetz.) W. Sm. (Text-fig. 10, 8-10).

This diatom occurred in the cultures of two soils. The valves were linear-lanceolate with capitate apices, and 11-12 carinal points in  $10\mu$ , distributed regularly along the margin of the valve; the striations were too faint to be made out. The length of the valves was about  $29$  to  $33\mu$ , and the breadth about  $4\mu$ .

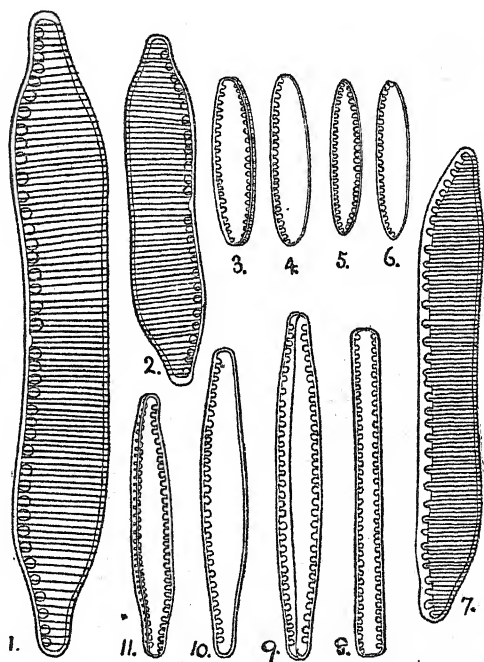
var. *fenticola*, Grun. (Text-fig. 10, 11).

A diatom very closely resembling this variety was found in one soil. The valves had strongly capitate apices and there were 15 carinal points in  $10\mu$ , while their length was about  $15\mu$  and breadth  $3.5\mu$ ; the striations could not be made out clearly. The valves, however, seemed to be slightly sigmoid, but this character, taken in conjunction with the others, does not seem sufficient to justify the separation of this form from the above variety.

16. *Nitzschia inconspicua*, Grun. (Text-fig. 10, 3-6).

A number of very minute forms of *Nitzschia*, never exceeding  $16\mu$  in length, were found in six of the soils, and though not in exact agreement with *N. inconspicua*, Grun., they should probably be regarded as forms of this species.

The form most nearly in agreement with the description of this species (Text-fig. 10, 3 and 4) was lanceolate with rounded ends and had 13 to 14 carinal points in  $10\mu$ ; the striations were too faint to be seen. The valves were  $15$  to  $16\mu$  long and about  $4\mu$  broad.



TEXT-FIG. 10. Soil-diatoms III. 1 and 2, *Hantzschia amphioxys*, (Ehr.) Grun.; 3-6, *Nitzschia inconspicua*, Grun.; 7, *Nitzschia obtusa*, (W. Sm.) var. *scalpelliiformis*, Grun.; 8-10, *Nitzschia Palea*, (Kuetz.) W. Sm.; 11, *Nitzschia Palea*, var. *fenticola*, Grun. All figs.  $\times 1435$ .

A second form (Text-fig. 10, 5 and 6) had more linear valves with abruptly tapering rounded apices, and was about 14 to 15  $\mu$  in length by 3  $\mu$  in breadth. There were 15 to 16 carinal points in 10  $\mu$  and the striations were too indistinct to be determined.

Both of these forms differ in the number of carinal points from that originally described for the species, but the agreement is so close in other respects that it seems advisable to regard them merely as variable forms of the same species. No previous record has been made of its occurrence in the British Islands.

### Chlorophyceae.

#### 1. *Chlamydomonas communis*, Snow (Text-fig. 11, 1-7).

A species of *Chlamydomonas* was found in the cultures of five of the soil-samples which agreed very closely with *C. communis*, Snow. This species, which has not previously been recorded for this country, was regarded by Snow as a plankton-species, but the characters of the form seen in the cultures resemble those of Snow's species so closely that it seems probable that the species has a wider distribution than has previously been thought. The adult motile cells were subcylindrical or oval with a pointed anterior end, 11.5 to 13  $\mu$  long and 6 to 7  $\mu$  broad, with a thin wall and a continuous bell-shaped chloroplast often raised into more or less prominent cushion-shaped lobes; there was a single large pyrenoid in the centre of the cell. The pigment-spot was inconspicuous and near the anterior end of the cell. Zoogonidia, 4.5  $\mu$  broad by 9  $\mu$  long, were formed by the longitudinal fission of the mother-cell contents into four parts, and were identical in structure with the adult cells. Very rarely a slightly oblique fission was observed (Text-fig. 11, 7), but in this case both planes of fission were parallel to the same axis of the cell.

#### 2. *Chlamydomonas pluristigma*, n. sp. (Text-fig. 11, 8-14).

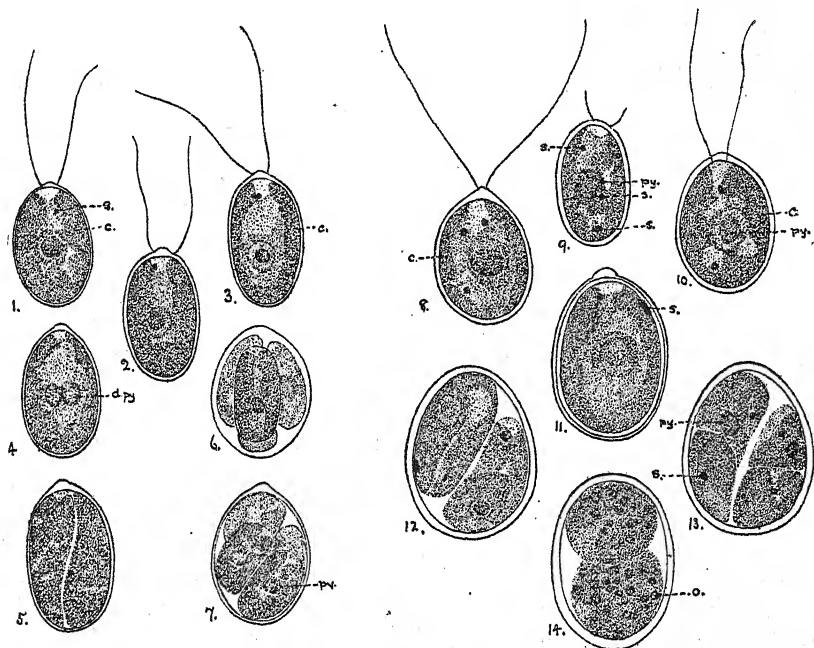
This species was found in a single soil only, but its characters differed considerably from those of any previously described species. The adult cells were oval, with usually a pointed anterior end; they were 13 to 16  $\mu$  long by 9.5 to 11  $\mu$  broad and possessed a comparatively thick cell-wall. The chloroplast was bell-shaped and lined practically the whole cell-wall except at the anterior end, but it was occasionally perforated (Text-fig. 11, 9 and 10) and the inner surface was frequently raised into cushion-shaped lobes. There was a single large pyrenoid near the centre of the cell, and two or three pigment-spots variously disposed in the cell, one near the anterior end being much larger than the rest. Zoogonidia, 7  $\mu$  broad by 12  $\mu$  long, were formed by one oblique and one transverse division of the contents, and were similar to the adult cells.

The following is a diagnosis of the species: Zoogonidiorum tegumentum satis crassum, apice in papillam hyalinam provectum. Cellulae ovaes vel

ovoideae, 13-16  $\mu$  longae, 9.5-11  $\mu$  latae, ciliis binis circa  $1\frac{1}{2}$ -plo longioribus praeditae; stigmatibus 2 vel 3, disciformibus, varie dispositis. Chlorophorum ampullaceum in lobos pulviniformes intus projectum, nonnunquam perforatum, uno magno pyrenoide media parte instructum.

Propagatio fit divisione cellularum matricialium, altera obliqua altera transversa, in 4 zoogonidia.

*Hab.* in solo culto, Sedgley (Staffs.).



TEXT-FIG. 11. 1-7, *Chlamydomonas communis*, Snow; 1-3, motile vegetative cells; 4, resting cell in which division is about to take place; 5 and 6, longitudinal fission into four zoogonidia; 7, oblique fission; c., chloroplast; py, pyrenoid; d.py., dividing pyrenoid; s., pigment-spot; o., oil globules; 8-14, *Chlamydomonas pluristigma*, n. sp.; 8 and 9, motile cells with three pigment-spots; 10, ditto with two pigment-spots and perforated chloroplast; 11, resting cell with two pigment spots; 12, oblique fission of cell into two, followed by 13, transverse fission into four; 14, transverse fission of cell.  $\times$  Circ. 1430.

### 3. *Coccomyxa Solorinae*, Chod., forma.

In seven of the soil-samples a species of *Coccomyxa* appeared which agreed more clearly with *C. Solorinae* than with any other described species, but which differed from it in the relative length and breadth of the cells. The cells were oval with rounded or more rarely pointed ends, 6 to 7  $\mu$  long by 3 to 3.5  $\mu$  broad, and contained a single parietal chloroplast but no pyrenoid. The division of the cell took place at first in an almost transverse plane, but this later became oblique, and the daughter-cells were liberated by the dissolution of the mother-cell wall to form a thin mucilaginous

envelope in which the young cells remained embedded for some time. In some cases a second division took place before the separation of the young cells, so as to produce groups of four cells enclosed in a common mucilaginous envelope.

This species has not previously been recorded for the British Islands.

4. *Trochiscia aspera*, (Reinsch) Hansg.

This very widely distributed species was obtained from thirty-four different soil-samples. In many cases the form observed was exactly typical of the species; but this species showed perhaps more than any other a tendency to change as the result of cultural conditions. The cells were spherical or subspherical, 14 to 20  $\mu$  in diameter or more rarely up to 33  $\mu$ , with several parietal chloroplasts, some of which contained a single pyrenoid, in each cell. In the normal form the cell-wall was ornamented with prominent denticulations connected together by low ridges, but in the cultures this character tended to be suppressed, the denticulations becoming less prominent and the ridges almost absent; there was, however, no reason for thinking that these cells were other than abnormalities, since every gradation could be found between the two extremes. A palmelloid state exactly similar to that figured by West<sup>1</sup> was frequently observed. Multiplication was by the production of non-motile gonidia.

5. *Trochiscia hirta*, (Reinsch) Hansg.

In general features and in its life-history this species agreed completely with the last, but it differed in the character of its cell-wall. Instead of having comparatively few prominent denticulations, the wall was ornamented with very numerous minute spines so closely crowded together as to give it an almost granular appearance. It occurred in two soils.

6. *Ankistrodesmus falcatus*, (Corda) Ralfs, forma *terrestris* (Text-fig. 2).

The cells of this form, found in only one soil, were lunate, but somewhat narrow, with acute apices. They were 2 to 2.5  $\mu$  broad by about 18  $\mu$  long, and contained a single chloroplast, usually devoid of a pyrenoid but very occasionally containing one. Multiplication was observed by the production of four or eight autospores which, on the gelatinization of the mother-cell wall, usually remained loosely attached together by one end (Text-fig. 2, d). The further production of autospores in the same way gradually gave rise to the formation of loose and somewhat irregular colonies, as shown in Text-fig. 2, e.

7. *Dactylococcus bicaudatus*, A. Br.

This species was found in one soil. The cells were lunate with greatly prolonged apices, and contained a single rather small chloroplast devoid of a pyrenoid. The length of the cells was 20 to 25  $\mu$ , and the breadth 3.5 to 5  $\mu$ . Multiplication was observed to occur by the production of four autospores.

<sup>1</sup> West, G. S.: British Freshwater Algae. Cam. Biol. Series, 1904, Fig. 82, F.

8. *Dactylococcus dispar*, W. and G. S. West.

This curious form was observed in the cultures of one soil. The cells varied considerably in shape, being either lunate or somewhat distended and irregular. The two extremities of the cells were unequally developed, one often being rounded and the other acute or acuminate and sometimes bent, but there were no prolonged apices as in the last species. The cells were 13 to 19  $\mu$  long and 2.5 to 6  $\mu$  broad, with a parietal chloroplast but no pyrenoid. Multiplication took place by the formation of four autospores.

9. *Chlorococcum humicola*, (Naeg.) Rabenh.

This species was found in the cultures of every soil but one. The cells contained a single parietal chloroplast with a variable number of pyrenoids, and were observed to multiply either by the formation of zoogonidia or by means of aplanospores which gave rise to a palmella-state. A full account of the life-history and cytology of this alga has been given in a separate paper,<sup>1</sup> however, and further details are unnecessary here.

10. *Chlorochytrium paradoxum*, (Klebs) G. S. West.

This alga has also been fully described elsewhere.<sup>2</sup> It occurred in three different soils and agreed in every detail of its life-history with the description given by Klebs. The chloroplast consisted of a small spherical axial portion from which radiated outwards in every direction variously lobed and branched arms, the ends of which flattened themselves against the cell-wall.

11. *Vaucheria hamata*, (Vauch.) Lyngb.

The filaments of this species, which was identifiable in only one soil, were 35 to 39  $\mu$  broad. The oogonia were produced singly on the end of lateral branches about 65  $\mu$  long, and were subspherical, about 56  $\mu$  long by 62  $\mu$  broad, with very thick walls. Each antheridium grew on the end of a filament branching out from the lower part of the oogonial filament and was about 18  $\mu$  in diameter and strongly curved.

The dimensions of this form are somewhat small for the species in every respect, but the mode of growth and general characters leave no doubt as to its identity.

12. *Vaucheria* sp.?

In four other soils vegetative filaments of *Vaucheria* were observed, but in none of them were reproductive organs to be found, and consequently identification was impossible. In two cases the filaments almost exactly resembled those of the last species, but in the others they were usually wider, up to 60  $\mu$  in diameter.

<sup>1</sup> Bristol, B. M. : On the Life-history and Cytology of *Chlorococcum humicola*, (Naeg.) Rabenh. Journ. Linn. Soc., Bot., vol. xlv, July, 1919.

<sup>2</sup> Bristol, B. M. : A Synopsis of the Genus *Chlorochytrium*, Cohn. Journ. Linn. Soc., Bot. (inédit).

13. *Ulothrix subtilis*, Kuetz.

The normal form of this species appeared in the cultures of five soils. The cells were 4 to 5  $\mu$  broad and about the same length. After some time in the cultures the filaments were observed to break up into fragments consisting of a dozen or more cells, a character common to practically all of the filamentous forms growing in the cultures.

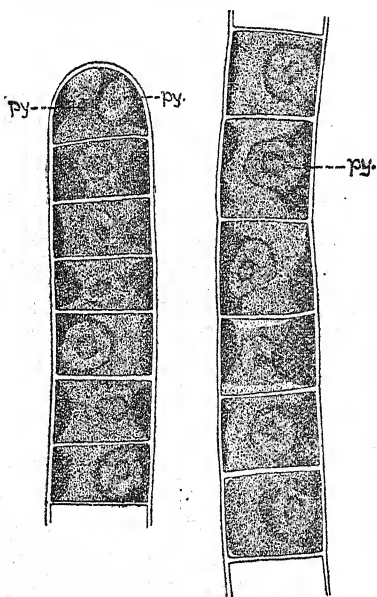
var. *variabilis*, (Kuetz.) Kirchn.

This variety of the species occurred much more frequently than the normal form, having been observed in twenty-seven soils. The cells were usually 8 to 9  $\mu$  broad and varied in length from 6  $\mu$  to about 17  $\mu$ . There was a single parietal chloroplast extending practically the whole length of the cell and about two-thirds of the way round, and containing as a rule a single large pyrenoid in which the separate starch-plates could frequently be seen. In the winter months, however, the pyrenoid sometimes was not visible. After some time the filaments were observed to break up into

fragments containing a comparatively small number of cells; this was effected by the rounding off of the end walls of two adjacent cells.

14. *Ulothrix tenuissima*, Kuetz.  
(Text-fig. 12).

In one of the soils a species of *Ulothrix* was observed which differed from *U. tenuissima* only in the number of its pyrenoids. The cells were 18  $\mu$  broad and 10 to 17  $\mu$  long, with a firm thin wall. The chloroplast extended practically the whole length of the cell and about three-quarters of the way round the wall, and contained a single large pyrenoid in which it was sometimes possible to detect the separate starch plates. In a single cell only, viz. in the end cell of the filament figured, there were two pyrenoids as has been described by Heering for this species.



TEXT-FIG. 12. *Ulothrix tenuissima*, Kuetz.; py., pyrenoid.  $\times 825$ .

15. *Stichococcus bacillaris*, Naeg.

The cultural conditions were such that the filamentous form of this species was very rarely observed, and such filaments as did occur consisted of not more than three cells.

The alga thus entered into a unicellular condition, the cells being rectangular with slightly rounded ends, about 6 to 8  $\mu$  long and 2 to 2.5  $\mu$  broad, with a parietal chloroplast devoid of a pyrenoid extending over about half of the surface of the cell. The species was found in four soils.

16. *Stichococcus nitens*, (Klebs) = *Hormidium nitens*, Menegh. em. Klebs.

In the cultures of one of the soils there appeared an alga which agreed in its characters very closely with *Hormidium nitens*, Menegh. em. Klebs, but the filaments were observed to be arranged more or less parallel to one another to form an expanded stratum, and were often completely disarticulated into single cells. Rarely short filaments were formed consisting of 2 to 4 cells, and sometimes long normal filaments could be observed. This mode of growth is entirely that of a *Stichococcus*, and its occurrence in a form that has previously been regarded as a species of *Hormidium* rather helps to indicate that the latter genus is based on somewhat unstable characters. The extraordinary confusion existing among the species of *Ulothrix*, *Hormidium*, and *Stichococcus* makes it wellnigh impossible to distinguish between *Hormidium* and *Ulothrix* on the one hand and between *Hormidium* and *Stichococcus* on the other; hence, pending further investigation, it seems best to regard the present form as a species of *Stichococcus* rather than of *Hormidium*. The cells were 5 to 6  $\mu$  broad and 8 to 15  $\mu$  long, frequently about 9  $\mu$ . The single parietal chloroplast extended usually from end to end of the cell and about two-thirds of the way round, and contained a single pyrenoid, or two in those cells about to divide. Disarticulation of the filaments was effected by the splitting apart and rounding off of the transverse walls of adjacent cells.

17. *Gongrosira terricola*, n. sp. (Plate II).

This new species of *Gongrosira*, found in soil-samples from three widely separated localities, differs from all others previously described in its terrestrial habit and in the basal or more rarely intercalary position of its zoogonidia. In the latter character it most closely resembles *G. Codiolifera*, Chod., in which the zoogonidia are intercalary in the upstanding filaments; but the absence of a limy incrustation establishes it as separate from this species independently of the position of the zoogonidia.

The stratum is expanded, and the creeping filaments are closely interwoven to form a pseudo-parenchymatous disc of distended cells. The upstanding filaments are usually comparatively short and tapering with obtuse apices; they are irregularly branched, either in small tufts (Pl. II, Figs. B and G) or with false dichotomy (Figs. D and F). The basal cells are swollen, 11 to 16  $\mu$  in diameter. Those of the upstanding filaments are subcylindrical or distended, and may be irregular on account of a tendency to branch; they are usually 10 to 18  $\mu$  long and 6 to 14  $\mu$  broad, with a single band-shaped or irregular chloroplast containing usually one pyrenoid, rarely two, or sometimes none. The zoogonidia are generally basal (Figs. A and H), but more rarely may be intercalary in the upstanding filaments. They are usually subspherical or irregular, 14 to 18  $\mu$  in diameter or occasionally less, opening by means of a lateral pore to set free a large number of biciliate zoogonidia.



The following is a diagnosis of this new species :

*G. terricola*, in solo culto vigens, stratum expansum efformans ; thalli pars inferior e filamentis densis ramosis, cellulis tumidulis subglobosis vel irregularibus constat, pars superior e filamentis erectis fasciculato- vel pseudodichotomo-ramosis apicem versus angustioribus, cellulis subcylindricis tumidulis vel irregularibus. Cellulae inferiores 11 to 16  $\mu$  crassae, cellulae filamentorum erectorum 6 to 14  $\mu$  latae, 10 to 18  $\mu$  longae, cellulae apicales obtusae non attenuatae, omnes chromatophoro irregulari parietali, pyrenoidibus singulis (rarissime binis) vel nullo instructae.

Zoogonidia basalia vel intercalaria subglobosa vel ovoidea, 14 to 18  $\mu$  crassa, ore minuto laterali dehiscentia.

*Hab.* in solo culto Kettering (Northants), Baggeridge (Staffs.), Tisbury (Wilts.).

18. *Tribonema bombycinum*, (Ag.) Derb. and Sol. (Text-fig. 5).

This species was found in the cultures of four different soils, and in most cases was quite typical in character, with slightly tumid cells, 8.5 to 9  $\mu$  broad by 18 to 21  $\mu$  long, and cell-walls breaking into H-shaped pieces in the fragmentation of the filament. Multiplication by means of zoogonidia was not observed in the cultures, but aplanospores were formed as described at the end of Section III of this paper.

19. *Bumilleria exilis*, Klebs. (Text-fig. 1).

This alga, though never previously recorded for the British Islands, was found in forty of the soils, and is evidently a typical soil-species, since it was originally described by Klebs from a very similar habitat. Its appearance in the cultures seemed to be periodic; its growth gradually attained to a maximum during the first eight months of the investigation, and in May, 1916, it was the dominant form present in many of the cultures. From that time onwards it gradually disappeared until in the early months of 1917 only a few isolated filaments could be found; while six months later it had again increased in quantity in many of the cultures, though the second maximum never attained to the height of the first.

The cells of the filaments were almost rectangular, with slightly tumid walls, and were usually about 4.5  $\mu$  broad. They varied in length from 10 to 15  $\mu$ , sometimes less, and contained a variable number of small, parietal, yellow-green chloroplasts, four in the shorter cells and 6 to 12 in the longer ones; starch was never observed in them, but small globules of oil were often present.

Multiplication took place most frequently by a fragmentation of the filament, either through the breaking of the wall of one of the cells into H-shaped pieces (Text-fig. 1, *d*), or more frequently through the splitting apart of the end walls of two adjacent cells (Text-fig. 1, *b*), and the consequent formation of knee-bends. In a few cases the early stages of



zoogonidia-formation were observed, four zoogonidia being produced within a cell, but these were never seen to acquire cilia or to escape from the cell, and it is possible that the cultural conditions were such as to preclude this form of multiplication.

## VI. SUMMARY.

The investigation by means of water-cultures of forty-four samples of soil from widely separated localities has shown that there is a widely distributed ecological plant-formation in cultivated soils consisting of moss protonema and algae. The most important algae in this formation are: *Hantzschia amphioxys*, (Ehr.) Grun., *Trochiscia aspera*, (Reinsch) Hansg., *Chlorococcum humicola*, (Naeg.) Rabenh., *Bumilleria exilis*, Klebs and to a less degree *Ulothrix subtilis*, Kuetz., var. *variabilis*, (Kuetz.) Kirchn. Other species of typical soil-algae occurring somewhat less frequently give rise to smaller plant-associations within this formation.

The total number of species and varieties found in these soils is sixty-four—20 Bacillarieae, 24 Myxophyceae, and 20 Chlorophyceae. The soil-samples had all been subjected to complete desiccation for 4 to 26 weeks before being placed in the cultures; hence these species could be expected to withstand any period of drought that might occur naturally.

It seems likely that this extensive algal formation must be of considerable economic importance in the biology of the soil.

Six new species or varieties are described, sixteen species already described are newly recorded for the British Islands, and a number of new or interesting stages are depicted in the life-histories of certain species already known, especially in connexion with the germination of the spores of some blue-green algae. The final section of the paper contains a short account of each of the species found in the cultures.

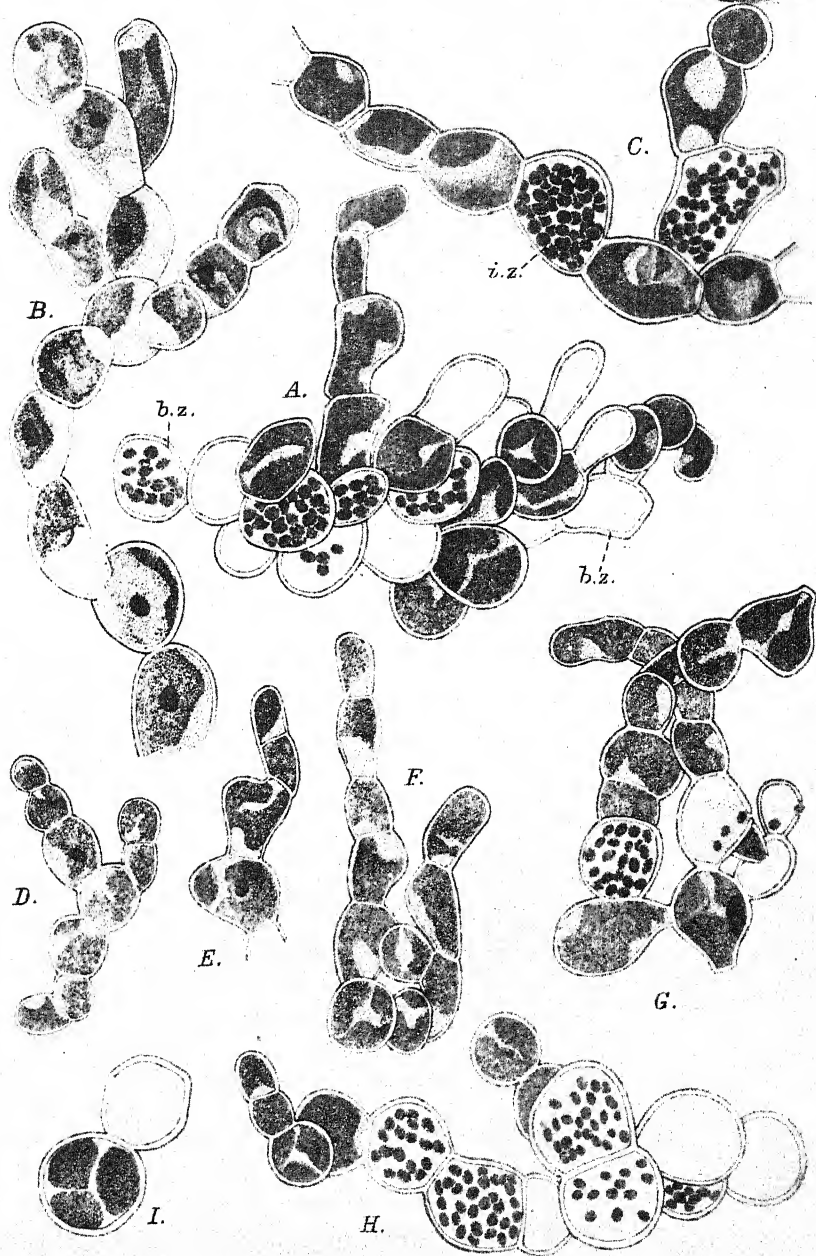
In conclusion, the writer desires to express her thanks to a number of friends who assisted her in making the collection of soil-samples. In particular she wishes to acknowledge her indebtedness to Professor G. S. West for his valuable help, especially in the identification of the species, and for his unfailing encouragement throughout the course of the investigation.

DESCRIPTION OF PLATE II.

Illustrating Miss B. M. Bristol's paper on the Alga-Flora of some Desiccated Soils.

All figures  $\times 825$ .

- A. Part of pseudo-parenchymatous base of thallus, with a single upstanding filament and numerous basal zoogonidangia (*b.z.*).
- B. Part of upstanding filament, showing tufted branching.
- C. Ditto, showing intercalary zoogonidangia (*i.z.*).
- D-F. Tapering apical branches, showing false dichotomous branching and irregular chloroplasts.
- G. Part of thallus, showing tufted branching and intercalary zoogonidangia opening by a lateral pore.
- H. Creeping filaments with basal zoogonidangia.
- I. Early stage in formation of zoogonidia.





# Observations on the Anatomy of Teratological Seedlings.

## II. On the Anatomy of some Polycotylous Seedlings of *Centranthus ruber*.

BY

DOROTHY BEXON, M.Sc.,

*University College, Nottingham.*

With nine Figures in the Text.

THE following study of polycotylous seedlings of *Centranthus ruber* was carried out in the hope of discovering, amongst the abundant material offered by this species, evidence corroborative of and supplementary to that obtained in the investigation of *Cheiranthus Cheiri* (9) as to the origin of polycotyly.

*Centranthus ruber* apparently yields a fairly high proportion of polycotylous seedlings, amongst which all the usual types of abnormality are included. Thus from about two square yards of soil on which self-sown seeds of *Centranthus* were germinating, a large number of polycotylous specimens were gathered, of which eighty-seven seedlings have been examined, these grouping themselves according to external characters as follows :

<i>Type of Seedling.</i>	<i>Number of specimens examined.</i>
Hemitricotyls	39
Tricotyls	38
Hemitetracotyls	5
Tetracotyls	4
Trisyncotyls	1

### STRUCTURE OF THE NORMAL SEEDLING.

*Centranthus ruber* has not apparently received any attention previously from the point of view of seedling anatomy, so that a short description of the normal seedling will not be out of place here.

The seedling is fairly robust, being usually from six to eight centimetres in length, whilst the cotyledons are of medium size, and are shortly petiolate. There is a slight tendency observable towards the formation of

a cotyledonary tube, but this is never pronounced and is entirely absent in many instances. Each cotyledon at its base contains a median collateral vascular bundle and two laterals, these, however, fusing with the central bundle before the cotyledonary node is reached. 'Rotation' commences usually in the base of the cotyledon, although it has been observed in some cases to be delayed until the apex of the hypocotyl is reached. The phloem mass of the median collateral bundles divides into two portions which separate slightly, whilst the protoxylem commences a gradual movement outwards, being first mesarch and later exarch in position. This is accompanied by a slight flattening of the vascular bundle, without however any wide separation of the metaxylem into two groups such as gives the characteristic 'double-bundle' appearance in *Cheiranthus* and many other forms. 'Rotation' is completed soon after the entrance of the bundles into the hypocotyl, but for a short distance the centre of the hypocotyl is occupied by a fairly large pith. This decreases in size and finally disappears, so that midway down the hypocotyl a solid diarch plate of xylem is produced, flanked by two groups of phloem.

*Hemitricotyls.* The hemitricotylous material examined included all possible stages of cotyledonary fission from the one extreme in which the lamina of the abnormal cotyledon showed no sign of splitting, this being indicated merely by the forking of the central vascular bundle, to the other in which the seedling was scarcely distinguishable from a tricotyl (Fig. 1).

As regards anatomical structure the hemitricotyls were divisible into three distinct groups according to the behaviour of their vascular bundles.

The first group, which included more than half the total number of seedlings examined, corresponded to the group which in the investigation of *Cheiranthus Cheiri* (9) was styled type *a*. In these seedlings each lobe of the bifurcated cotyledon was supplied by a collateral vascular bundle, the two bundles gradually approaching one another and finally fusing to form a single bundle, which in the transition region formed one pole of the diarch root (Fig. 2). The level at which the fusion of the bundles occurred showed great variation, and a series of forms was obtained in the simplest members of which the level of fusion was fairly high in the lamina, whilst in others it occurred in the upper and lower portions of the cotyledon petiole, and in the extreme cases at the apex of the hypocotyl.

It is noteworthy, however, that the level of fusion of the vascular bundles bore no relation to the extent of the split in the cotyledon, for in three seedlings in which the fission extended for one-third, one-half, and three-quarters of the length of the cotyledon respectively, the vascular bundles in all cases fused in the base of the petiole, whilst an even more extreme instance was found in two seedlings in which, while the lamina showed no sign of fission, the bundles did not fuse until the point of entry into the hypocotyl.

The second group of hemitricotyls, comprising eleven seedlings, conformed to the type of polycotylous seedling to which the name type  $\beta$  has been given. In these the two bundles of the bifid cotyledon entered the hypocotyl quite independently and rotated in the normal way, so that

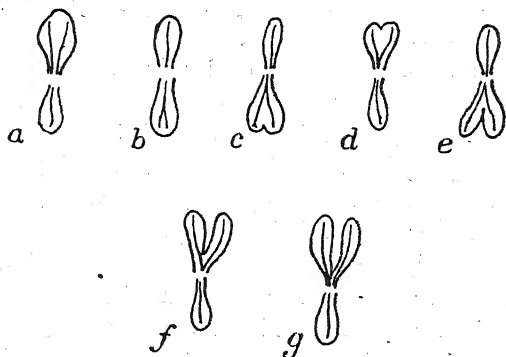


FIG. 1. *a-g*. Hemitricotylous seedlings showing various stages in the division of the abnormal cotyledon.

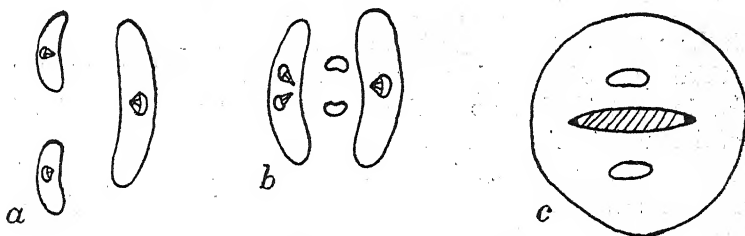


FIG. 2. *a-c*. Hemitricotyl type  $\alpha$ .

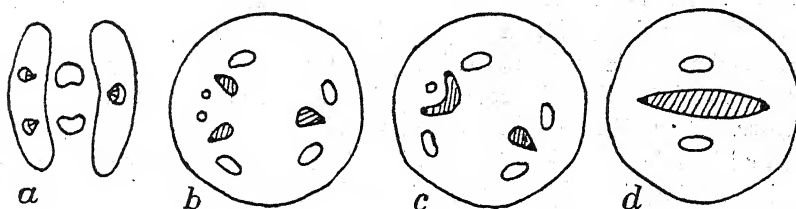


FIG. 3. *a-d*. Hemitricotyl type  $\beta$ . Diagrams *b*, *c*, and *d* show initiation of triarchy and reduction to diarchy by fusion.

a triarch stage obtained in the upper part of the hypocotyl, this, however, being subsequently reduced in all cases to diarchy. In about half the seedlings of this type the two bundles supplying the bifid cotyledon entered the hypocotyl in close proximity to one another, and immediately after rotation the adjacent half-phloem groups fused, this being followed by the fusion of the adjacent groups of metaxylem (Fig. 3). At a lower level the

phloem groups occupying the plane of bundle symphysis disappeared and complete fusion of the xylems followed, the typical diarch structure being produced. In other cases, however, a definite triarch structure was established at the apex of the hypocotyl, showing three widely separated xylem groups, and this condition persisted frequently down to a low level in the hypocotyl, or even into the root.

The reduction from triarchy to diarchy was accomplished in the majority of seedlings by the gradual approximation and subsequent fusion of two of the xylem arms, this being accompanied by the disappearance of the phloem group lying between the fusing arms (Fig. 3). In one case, however, after the xylem arms had approached one another quite closely, and were almost on the point of fusing, the protoxylem of one xylem mass disappeared, so that only the metaxylem took part in the fusion process.

It must be noted that there was no connexion whatever between the extent of fission of the cotyledon and the level at which the reduction to diarchy took place, since reduction occurred at the top of the hypocotyl in seedlings the abnormal cotyledon of which split in one case only for one-third of the length of the lamina, and in another instance almost to the base of the petiole, whilst reduction occurred in the root of another seedling in which the cotyledon showed only a slight apical cleft.

A third type of hemitricotyl remains to be described, this being of particular interest in that no similar phenomenon was met with in the course of the investigation of *Cheiranthus* seedlings, nor can any record of such structure be found in the literature dealing with polycotily in Angiosperms. The nearest approach to such a structure is found in the gymnospermous seedlings described by Hill and de Fraine (6), but the correspondence is not exact. In this type, of which four examples, exhibiting all degrees of fission of the cotyledon, have been examined, one of the bundles of the abnormal cotyledon appeared somewhat smaller than either its fellow or the bundle of the normal cotyledon, and on entering the hypocotyl only the two larger bundles rotated, whilst the third retained its collateral structure. At a fairly early stage the endarch protoxylem elements of the abnormal bundle disappeared, the metaxylem elements alone appearing at one side of the diarch plate formed by the two normal bundles (Fig. 4). Meanwhile the half-phloem groups arising from the sides of the two normal bundles adjacent to the collateral bundle fused with the phloem of the latter and produced one long band of phloem. At a later stage there was a movement inwards of the metaxylem elements so that they appeared almost connected with the diarch plate. In the lower region of the hypocotyl and in the root a typical diarch structure obtained.

*Tricotyls.* Of the thirty-eight tricotylous seedlings examined, only two belonged to the type  $\alpha$  group in which one of the cotyledons possessed collateral bundles, which on entering the hypocotyl rotated towards one



another and formed one pole of the diarch root. One of these seedlings showed two epicotyledonary leaves, whilst the other showed a whorl of three members.

In the majority of the seedlings the vascular bundle forming the midrib of each cotyledon commenced to rotate in the base of the cotyledon petiole or at the apex of the hypocotyl, a triarch stage being established, followed in most cases by a reduction to diarchy. In about half the seedlings the reduction occurred at a fairly high level in the hypocotyl, whilst in other cases it was delayed until the lower half of the hypocotyl or the root was

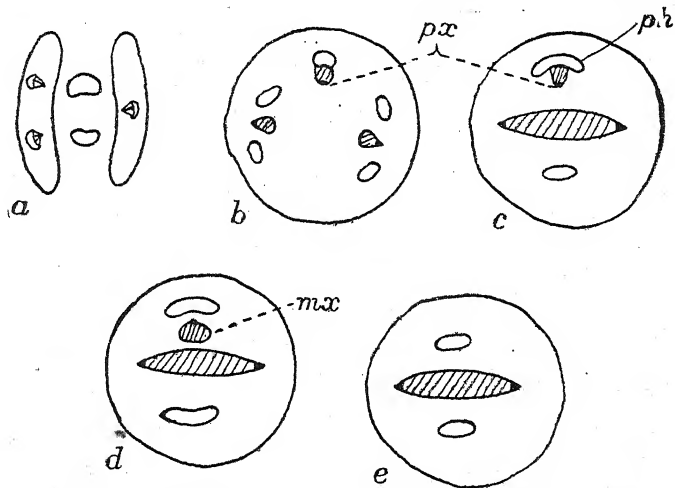


FIG. 4. *a-e*. Hemitricotyl in which the bundle supplying one lobe of the abnormal cotyledon retains its collateral structure. Diagrams *b* and *c* show the abnormal bundle with its endarch protoxylem, *px*. In *c* the phloem of the abnormal bundle has fused with the half-phloem groups on either side to form one long band, *ph*. Diagram *d* shows stage at which the metaxylem only of the bundle is present.

reached. Finally, in a few seedlings triarchy persisted throughout the whole seedling, but this condition was far less common than in *Cheiranthus*, where more than half the tricotyts examined showed persistent triarchy.

The reduction from triarchy to diarchy was accomplished by one of two methods, either by the gradual approximation and fusion of two xylem arms, this being accompanied by the disappearance of the intervening phloem group, or by the disappearance of one of the three xylem arms, followed by the fusion of the two phloem groups which lie on either side of it. Of the two methods the second was by far the more frequent, reduction by fusion of xylem arms being comparatively rare. In one only was there a condition intermediate between disappearance and fusion. In this instance two of the vascular bundles entered the hypocotyl in close proximity to one another and rotated in the usual manner, this being quickly followed by the disappearance of the median phloem group and a further approximation of

the two xylem groups. Before fusion was complete, however, the protoxylem elements of one xylem mass disappeared, so that only the metaxylem fused with the neighbouring group.

In the tricotyls as in the hemitricotyls a third type of seedling must be distinguished, this type being characterized by the fact that one of the three vascular bundles on entering the hypocotyl retained its collateral structure, whilst the other two bundles proceeded to undergo rotation and form a diarch plate. The protoxylem of the collateral bundle disappeared soon after its entry into the hypocotyl, and in one or two cases there was a movement of the metaxylem elements towards one of the xylem poles before their final disappearance. It was noticeable also that in some instances the cotyledon supplied by the collateral bundle was smaller than either of its fellows. In the majority of tricotylous seedlings the number of epicotyledonary leaves in the first whorl was three, but in a few cases two only were present, and in two seedlings one of the two epicotyledonary leaves was bifurcated.

*Hemitetracotyls.* Of this class five seedlings only have been examined, and since these present several features of interest they will be described separately.

*Seedling A.* Each cotyledon showed an apical notch which was slightly deeper in the one case than in the other (Fig. 5 (3 *a* and 3 *b*)). Each lobe of the cotyledons was supplied by a collateral vascular bundle, the pairs of bundles uniting in the one case in the petiole, and in the other at the junction with the hypocotyl, so that at the apex of the hypocotyl two bundles appeared which ultimately gave rise to a normal diarch plate.

*Seedling B.* This seedling as regards external appearance was almost identical with seedling A, since the two cotyledons each showed an apical cleft, and when the seedling was uprooted, it was classified without hesitation as a hemitetracotylous specimen (Fig. 5 (2 *a* and 2 *b*)). In anatomical structure, however, it showed striking differences from the condition described above. The two apparent cotyledons were each divided for a short distance only, but the median bundles of the associated lamina segments remained widely separated throughout the length of the 'cotyledon'. At the base of the cotyledon a lateral bundle appeared on the outer side only of each main bundle. Just above the cotyledonary node two distinct sets of plumular leaves were present, each set being dimerously arranged, but whilst one group showed two leaves only, in the other two pairs were visible (Fig. 6, *a*).

At the cotyledonary node each of the four main bundles rotated, and the opposed bundles, one from each 'cotyledon', united together to form a diarch xylem plate, so that the hypocotyl contained two widely separated diarch steles, this condition obtaining throughout its whole length (Fig. 6, *c*). In the root region, lateral rootlets were given off from each of the steles,

emerging simultaneously side by side from the adjacent poles of the xylem plates (Fig. 6, *d*).

As the tip of the root was approached the parenchymatous layers separating the two steles became reduced in number and finally disappeared entirely, so that the endodermal layers of the steles came into contact on

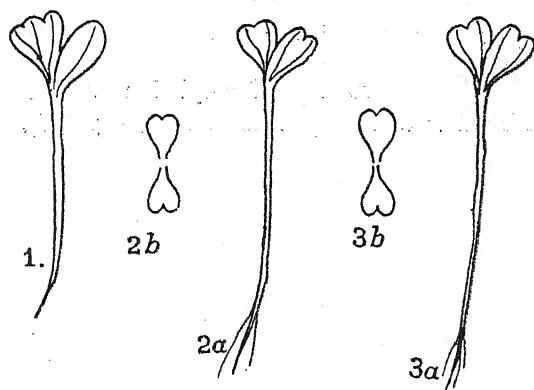


FIG. 5. 1, 2*a* and *b*, 3*a* and *b*. Hemitetracotylous seedlings. Seedling 2*a* and *b* is a 'twin' showing double structure.

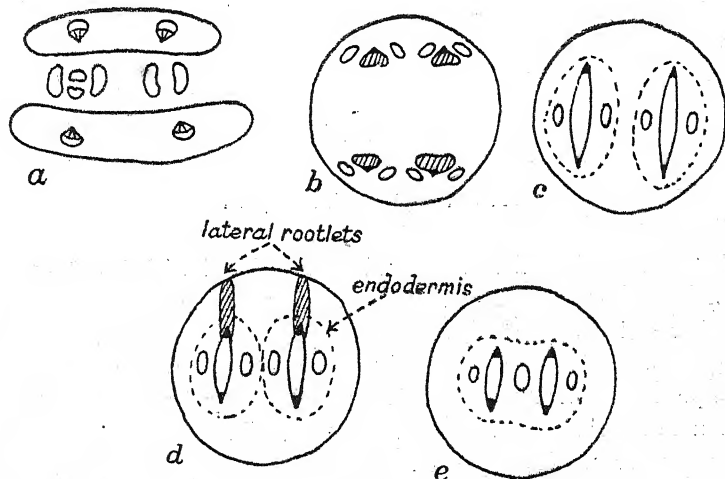


Fig. 6. *a-e*. Hemitetracotyl B. Diagrams illustrating double structure of 'twin' seedling.

their adjacent sides. Later still the contiguous portions of the endodermal layers disappeared, so that the adjacent phloem groups came into contact with one another, and became rather reduced in size. No further stage was shown in the seedling (Fig. 6, *e*).

*Seedling C.* In this instance the seedling possessed two normal

cotyledons, and a third which showed fission of the lamina for a short distance. Each half of this abnormal cotyledon was supplied by a collateral vascular bundle, the two bundles in spite of the early fusion of the halves of the lamina remaining distinct and widely separate throughout the whole cotyledon, and finally entering the hypocotyl quite independently. At the apex of the hypocotyl the four bundles were grouped in pairs, each pair consisting of one bundle from the split cotyledon, and one connected with a normal cotyledon.

The bundles of the two normal cotyledons commenced to rotate, followed by the bundles of the split cotyledon (Fig. 8, *b*). Bundle D was

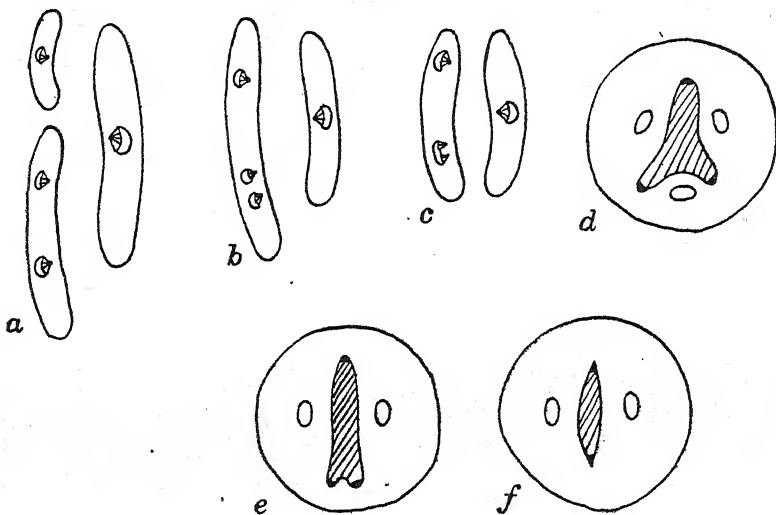


FIG. 7. *a-f*. Hemitetracotyl D.

somewhat smaller than the others and quickly fused with bundle B, so that a triarch stage resulted. Later the bundles C and A gradually approached one another and finally fused, the phloem group situated between the two xylem groups passing over and fusing with the other phloem group of C (cf. Compton (3), *Ulex europaeus*), so that ultimately the hypocotyl and root showed a normal diarch structure (Fig. 8). In this case, therefore, the vascular bundles which supplied the lobes of the bifid cotyledon fused not with one another but with the bundles A and B respectively which supplied the normal cotyledons.

**Seedling D.** This possessed two cotyledons, of which one presented a normal appearance, whilst the other was approximately twice the size of its fellow, and showed at its apex a slight notch and also a deeper cleft which extended for almost half the length of the lamina, each of the three lobes so produced being supplied by a vascular bundle (Fig. 7 *a*). The two

bundles supplying the lobes separated by the slight apical notch approached one another gradually and fused together in the lower portion of the lamina, whilst the vascular bundle so produced, and the bundle which supplied the third lobe of the cotyledon, entered the hypocotyl independently and proceeded to rotate so that a triarch structure obtained (Fig. 7, *d*). This persisted throughout the hypocotyl and the major portion of the root, but in the region of the root tip two of the xylem plates approached one another and finally fused, this being accompanied by the disappearance of the intervening phloem group (Fig. 7, *e* and *f*).

*Seedling E.* The last seedling of this type offered no points of outstanding interest. Both cotyledons showed slight apical fission, but whilst

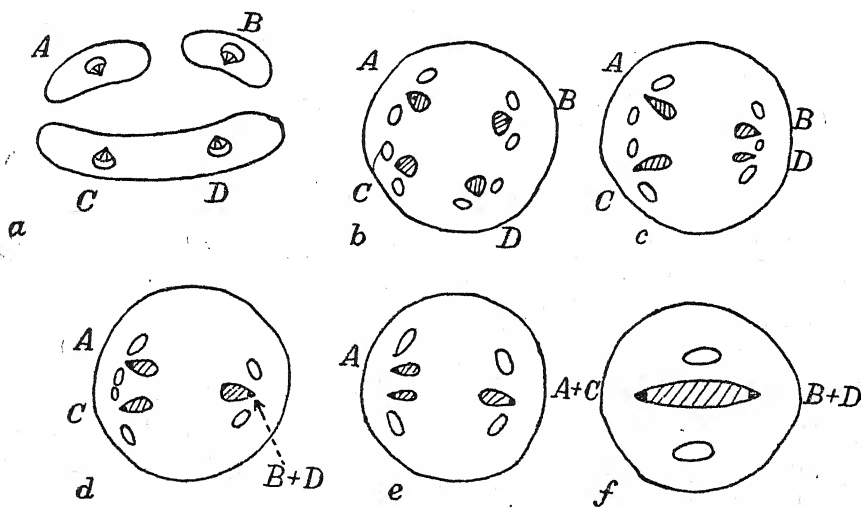


FIG. 8. *a-f*. Hemitetracotyl C. The bundles supplying the cotyledon lobes are lettered A-D. Diagrams *b*, *c*, *d*, show stages in fusion of bundles B and D. Diagrams *e* and *f* show fusion of A and C. Note that the fusing bundles supply lobes of distinct cotyledons, not the lobes of one cotyledon.

in one case the two collateral bundles fused at the base of the petiole, the bundles of the other cotyledon remained distinct throughout, and a fairly prolonged triarch stage ensued, this being followed later by reduction to diarchy through the dying away of one of the poles connected with the bifurcated cotyledon.

*Tetracotyls.* Only four examples of this type of polycotylly have been examined, and as these were all of type  $\alpha$  or type  $\beta$  or showed combinations of the types they only demand a brief reference.

In the simplest case the cotyledons were grouped in obvious pairs, union occurring between the corresponding vascular bundles at the apex of the hypocotyl, so that a normal diarch stele resulted.

In a second seedling the vascular bundles of two of the cotyledons, on

entering the hypocotyl, rotated and formed two root poles, whilst the remaining pair of bundles rotated towards one another at the entrance to the hypocotyl, and formed the third pole of the triarch root. Later one xylem group disappeared, diarchy obtaining throughout the remainder of the seedling.

The remaining seedlings showed a transitory tetrarch stage at the apex of the hypocotyl, this being quickly reduced to triarchy by the fusion of two of the xylem groups. Reduction to diarchy ensued in both seedlings, this being produced in the one instance by fusion of xylem plates, and in the other by the disappearance of a xylem plate.

*Syncotyls.* Syncotily, whilst not so frequent an abnormality as polycotily in *Centranthus*, is not by any means a negligible factor in the consideration of the seedling structure.

One of the seedlings examined may be termed a trisyncotyl, since it possessed three cotyledons the laminae of which were fused laterally for approximately half their length. Two of the cotyledons were of normal size, whilst the central one was considerably smaller (Fig. 9). Each of the three vascular bundles rotated on entering the hypocotyl, this being followed, however, by the speedy disappearance of the small bundle and the development of diarch structure.

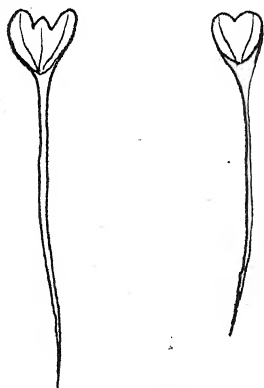


FIG. 9. Syncotylous seedlings.

Three other seedlings were examined in which the two cotyledons present were united laterally for a considerable portion of their length, so that in one case only a slight notch appeared at the apex of the cotyledon. Whilst these seedlings present interesting details in

their anatomy, these are not sufficiently relevant to be discussed here, but the seedlings are recorded as further examples of a tendency very common in *Centranthus*—the tendency towards fusion of distinct parts.

#### DISCUSSION.

In the investigation of *Cheiranthus Cheiri* (9) two modes of cotyledon increase were recognized: one type  $\alpha$ , in which the two cotyledons were produced by the qualitative division of the cotyledon apex, so that the vascular strand of each daughter cotyledon formed with its fellow one root pole, and the other type  $\beta$ , in which there was a qualitative division of the growing point so that the vascular strand of each daughter cotyledon gave rise to a root pole independently.

It will be obvious that the majority of the *Centranthus ruber* seedlings

examined show a behaviour strictly comparable with that found in *Cheiranthus Cheiri*, examples of type  $\alpha$  and type  $\beta$  being abundant, and in so far as this is the case the conclusions previously drawn are confirmed. There are, however, in some respects interesting points of difference between the two forms.

An examination of the material shows that there is in *Centranthus* a tendency towards the fusion of distinct parts, this showing itself in the attempts at the formation of a cotyledonary tube, in the existence of the di- and tri-syncotyls, and in numerous fusions, partial or more complete, of the cotyledon laminae among several groups of the polycotyls. Thus in hemitetracotyl C it seems highly probable that the bifid cotyledon has been produced as a result of a process of secondary fusion between two distinct cotyledons. The same feature is observable in hemitetracotyl D, and in many of the hemitricotylous seedlings, in which, as has been previously noted, there is frequently no relation between the level of fusion of the cotyledon halves and the level of union of the vascular bundles, so that the bundles may be distinct throughout the cotyledon, whilst there is only a slight apical notch visible in the lamina.

A similar interpretation may be placed upon the instances in which hemitricotylous forms show triarchy persistent for the major portion of the seedlings, and that this feature is not confined to *Centranthus* is shown by the fact that Hill and de Fraine (7) record a specimen of *Silene Schaftia* which although triarch throughout is secondarily dicotylous, doubtless through the complete fusion of two of the cotyledons, the double origin of the abnormal 'cotyledon' being indicated by its much larger size. It will be evident therefore that no seedling can be classified accurately solely by its external appearance.

Another feature of interest is found in those hemitricotyls and tricotyls in which one of the vascular bundles, on entering the hypocotyl, retains its collateral structure. This condition suggests comparison with the class of cotyledon styled 'subsidiary' by Hill and de Fraine (6), and allowing for the much earlier formation of the solid xylem plate in *Centranthus* the structure seems not unlike that described in some of those 'subsidiary' cotyledons.

From the description given by Hill and de Fraine it appears that they consider this type of cotyledon may be produced by either—

(1) A lateral splitting off of tissues from a normal cotyledon, a behaviour suggested by the structure found in *Pinus montana*, var. *gallica*, series B, and in *Larix europaea*, series C; or (2) a displacement of an epicotyledonary leaf to the cotyledonary level, as in *Cedrus Deodara*, series A, and *Pinus Pinea*, series C.

To these a third alternative may be added, namely, that the member is a cotyledon the vascular system of which is behaving anomalously.

In the *Centranthus* seedlings the somewhat smaller size of the collateral bundle together with the occasional reduction in size of the cotyledon lamina might be adduced in support of the first alternative, namely, that the abnormal cotyledon has arisen as a result of lateral fission; but the anatomical structure is difficult to reconcile with such an origin. All three cotyledons show the typical arrangement, that is, a median bundle flanked by two laterals, these fusing with the central one at the base of the petiole. The acceptance of this interpretation would therefore involve the assumption not only that the 'promoted' lateral had undergone downward prolongation into the hypocotyl, but also that new laterals had been developed in each of the two cotyledons resulting from the fission. Whilst the downward prolongation into the hypocotyl might easily result from the increased importance of the bundle when supplying a distinct cotyledon, the restoration of complete symmetry by the development of new laterals would be, in the absence of cognate evidence, an unwarranted assumption. It must be concluded, therefore, that the evidence available renders this alternative an extremely improbable one.

The possession of lateral bundles would accord with the second possible method of origin, namely, by the displacement of an epicotyledonary leaf, since these also typically possess a median bundle flanked by two laterals. The epicotyledonary whorl, however, presents a perfectly normal appearance consisting of two members (a condition found also in some of the normal tricotylys), whilst a further difficulty is presented by the fact that, since some forms are hemitricotylous and one secondarily dicotylous, it would be necessary to assume not only that the leaf had undergone displacement, but also that it had fused with one of the cotyledons.

It must be noted also that wherever among Angiosperm seedlings the strands of epicotylar leaves play any important part in the hypocotyl, they show rotation and are connected with a root pole, as is reported by Davey (5) in *Fuglans nigra*, and by Compton (2) in *Caesalpinia sepiaria* and *Pithecolobium Unguis-cati*. No signs of doubleness have, however, been observed in the vascular bundles of the epicotyledonary leaves in *Centranthus*.

As both the first and second alternatives seem to be excluded by the evidence at hand there remains only the third method of origin. The possession of laterals, and the frequent persistence of the metaxylem elements at least of the bundle throughout the major portion of the hypocotyl, accord with this interpretation, as does also a temporary slight division of the phloem in one instance, but no evidence as to the cause of the anomalous behaviour shown by the type  $\beta$  cotyledon is supplied by the material available.

There remains to be discussed the so-called hemitetracotylous seedling B, which showed double structure throughout. Twin seedlings of this type have been described by de Vries (10) in *Amaranthus speciosus*, *Datura*



*Stramonium*, and *Acer pseudoplatanus*, and also by Braun (1) in *Coelobogyne ilicifolia*, *Euonymus latifolius*, and a number of other species. In some instances the seedlings were fused together for only a short distance; others exhibited complete fusion of the hypocotyl and root, the origin however being indicated by a more or less deep cleft in the hypocotyl; others, again, presented the appearance of normal tetracotylous seedlings. In no case, however, were the cotyledons fused as in the *Centranthus* seedling.

Two possible explanations of the twinning phenomenon present themselves:

(a) We may assume that the early divisions of the embryo initial are quantitative in character, and that in this case the two daughter-cells have been separated, each developing into a complete seedling without, however, the connexion between the two being completely severed.

(b) It is possible that two distinct embryos may have been produced either in the same or in different embryo-sacs, these embryos becoming more or less completely fused together.

That the early divisions of the embryo are quantitative in character in the Coniferae seems to be clearly demonstrated by the fact that each cell of the embryo tier may independently produce an embryo, although usually only one of these develops to maturity, whilst in those forms such as *Sequoia* in which the embryo tier consists of one cell only this may divide by a longitudinal wall, and if the daughter-cells separate, two embryos may be produced (Coulter and Chamberlain (4)). Among Angiosperms only one case of this type has been reported. A. Braun (1), apparently quoting Hofmeister (8), stated that in *Loranthus europaeus* the terminal cell of the proembryo divides by two longitudinal walls, laid down at right angles to one another, and that each of these cells after further divisions may form an embryo. Usually, however, only one embryo matures, the production of two or more embryos being a rare occurrence.

Whilst in these cases fusion between the sister embryos is not recorded, such twinning is not unknown in the Animal Kingdom as a result of the partial separation of the early blastomeres in holoblastic ova.

It seems not impossible, therefore, that the 'twinned' seedlings of *Centranthus* and other forms may be produced in the manner suggested.

The second possibility remains that the 'twin' may have arisen by the fusion of two distinct embryos, these arising either in one embryo-sac or in separate embryo-sacs, or in distinct ovules which have undergone fusion. In this connexion it is interesting to note that Hofmeister records that in *Viscum album* the embryos produced in the one embryo-sac may fuse by their cotyledons. Braun also suggests that the twin seedlings of *Coelobogyne* may arise from the fusion of two distinct embryos, basing his suggestion on the facts that two or more embryos have been observed in one embryo-sac, and that the abnormal seedlings form a series leading from types in which

fusion occurs only for a very short distance along the hypocotyl, to others in which it is so complete as to produce an apparently tetracotylous seedling.

It must be pointed out, however, that the production of two or more distinct embryos may be accompanied by the splitting of one or more of them, as is shown in the Coniferae, and that when, as in the Animal Kingdom, twinning is known to be due to the separation of the two halves of a single embryo, fusion may be only partial.

Whilst both suggested methods of 'twinning' seem possible the lack of evidence as to ovular conditions in *Centranthus* forbids any decision as to the actual mode of origin of the seedling under discussion.

#### SUMMARY.

- (1) The vascular anatomy of a number of *Centranthus ruber* seedlings showing all stages of polycotyly from hemitricotyly to tetracotyly is described.
- (2) These, whilst agreeing in broad outline with the types of structure found in *Cheiranthus Cheiri*, are found to differ in—
  - (a) the frequency of fusions between cotyledons.
  - (b) the presence in some seedlings of a cotyledon the median bundle of which retains its collateral structure. The significance of these features is discussed.
- (3) The vascular anatomy of a 'twinned' seedling is described, the 'twinning' being due to—
  - (a) the fusion of distinct embryos, or
  - (b) the partial separation of the daughter-cells resulting from the quantitative division of the embryo initial.

I am indebted to Professor J. W. Carr, University College, Nottingham, who has granted every facility for the investigation, and also to Mr. H. S. Holden, University College, Nottingham, for his valuable help and criticism.

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## A New Species of *Uronema* from India.

BY

S. L. GHOSE, M.Sc.,

*Assistant Professor of Botany, Government College, Lahore, India.*

**With Fifteen Figures in the Text.**

OF the genus *Uronema*, Lagerh., only three species have been recorded up to now. *U. confervicolum*, Lagerh., and *U. simplicissimum*, (Reinsch) Lagerh., were described in 1887,<sup>1</sup> while *U. elongatum*, Hodg., was described in July, 1918.<sup>2</sup> In the beginning of March, 1919, I came across a species of *Uronema* which differed from all these three species. It was originally found growing on the walls of a narrow drain carrying dirty water from the kitchen in my house. The alga was taken in tubes in the original drain-water. In the laboratory it was washed in tap-water and examined. Numerous zoospores were seen to be given off, most probably on account of the change of environment. A few days afterwards fresh material was taken, and the same process was repeated. Again the result was the same. Some material was kept in a small jar filled with a mixture of tap- and the drain-water. This was placed near a window in the laboratory. In a week's time the walls of the jar were seen to be covered with young filaments produced from germinating zoospores.

The filaments are quite unbranched, and not enclosed in any mucous sheath. The mature ones sometimes reach a length above 7 mm. They are attached to the substratum by a rounded mucous disc found at the extreme tip of the basal cell. The basal cell shows a variety of forms. In mature filaments it is very long and attenuated, and is generally hyaline (Fig. 1). It may be straight or curved (Figs. 2 and 3). In young filaments it is much shorter and usually has a chloroplast, which, however, dies off as the filament grows (Fig. 4).

The apical cell also is, as a rule, gradually attenuated. Sometimes it is swollen at the base (Fig. 5). The tip is acuminate and usually slightly curved (Fig. 6). It may, however, sometimes be quite straight (Fig. 3) or very much curved (Figs. 7 and 8). The cell-wall in the apical part of the

<sup>1</sup> Lagerheim in Malpighia, I, fasc. xii, 1887.

<sup>2</sup> Hodgetts, W. J.: *Uronema elongatum*. New Phytologist, July, 1918.

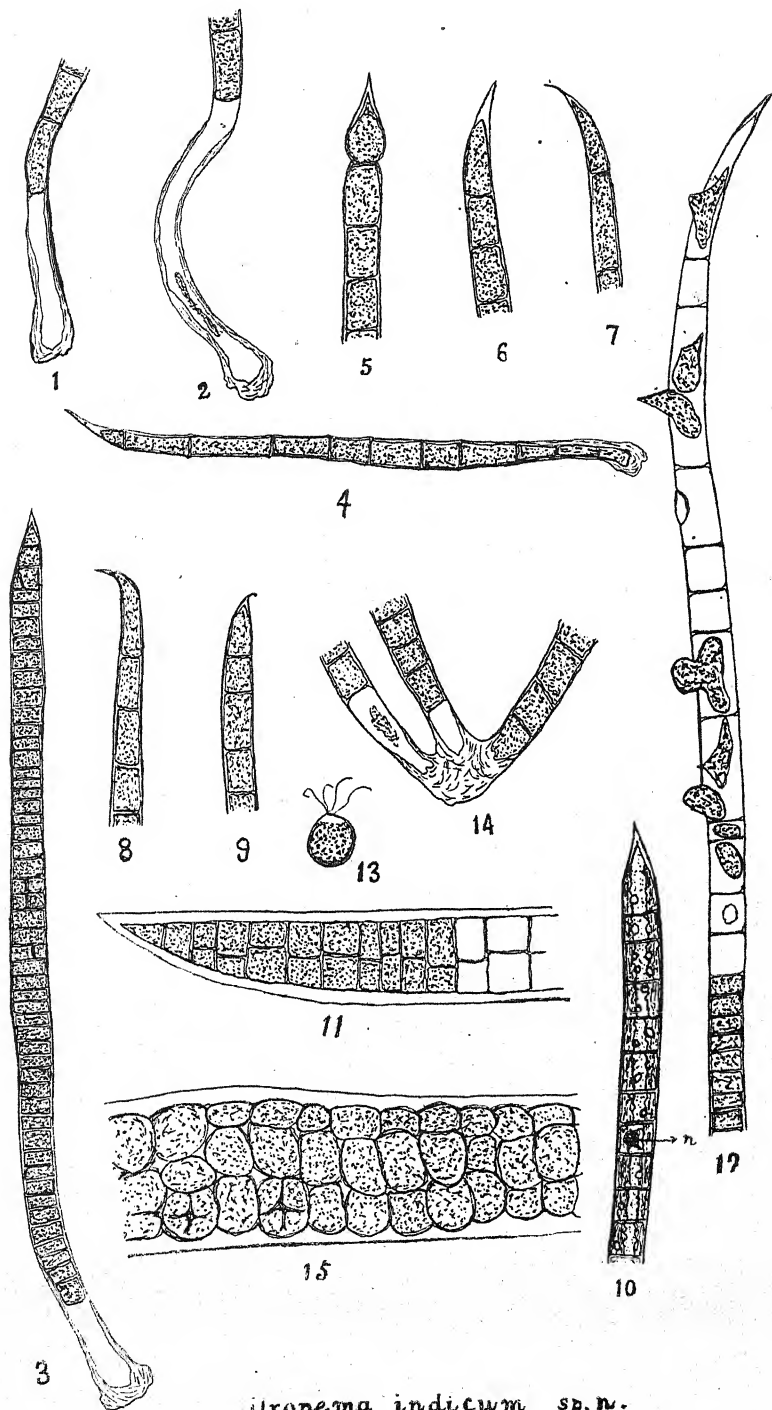
end cell was often found to be slightly thickened, and in a few cases the tip also ended in a dot-like thickening (Figs. 6 and 9).

The remaining cells are cylindrical and vary very much in size. In young filaments they are longer than they are broad, while in mature ones they are broader than they are long. At the time of zoospore formation, the width of the filament becomes considerably increased, sometimes two or three times the width found in the vegetative condition. The cells of a mature vegetative filament usually range between  $16\mu$  to  $22\mu$  in thickness and  $10\mu$  to  $18\mu$  in length (Fig. 10). A slight swelling is sometimes seen at the junctions of cells (Fig. 4). Each vegetative cell has a single band-shaped chloroplast, which runs through nearly the whole length of the cell and passes round about two-thirds of the cell circumference. Its edges are irregular and more or less incised. Pyrenoids up to four in number have been seen in some cells (Fig. 10). They are usually associated with numerous small grains of 'stroma starch'. These grains are more conspicuous in cells forming zoospores; in fact they seem to fill up the whole cavity of the cell at that time. Chloroplasts are very easily seen by staining the filaments with gentian violet. There is a single nucleus in each cell. It is not easily seen as it is generally covered by the chloroplast. Only rarely is it seen lying near the centre of the cell (Fig. 10, *n*).

The chief method of propagation is by 4-ciliate zoospores. In younger filaments the whole cell produces a single zoospore, but as the filament grows the cells form transverse and longitudinal septa, thus making the filament multiseriate (Figs. 3 and 11). Each of the cells thus formed produces one zoospore. The escape of zoospores is similar to that found in *Uronema elongatum*<sup>1</sup> (Fig. 12). Sometimes, however, it was observed that a zoospore while coming out through the pore became constricted and finally divided into two. Thus two zoospores were produced from one cell without a septum being formed at first. The zoospore is nearly rounded and about  $10\mu$  in diameter (Fig. 13). It is characterized by possessing abundant starch grains, which conceal the nucleus and the chloroplast. No pyrenoid was observed, perhaps also on account of its being concealed by the starch grains. Very soon after its emergence the zoospore becomes active and swims away. Germination takes place as in *U. elongatum*.<sup>1</sup> Sometimes two or three zoospores germinate together, and their basal mucous discs become confluent (Fig. 14).

The chief interesting points about this alga are its great length and great variation in size. Sometimes the filament is packed with zoospores and attains a thickness of  $40\mu$  (Fig. 15), while a young filament may be only  $10\mu$  in thickness. Fig. 3 shows a filament about  $13\mu$  in thickness, some cells of which are forming longitudinal septa also. Fig. 11 shows a part

<sup>1</sup> Hodgetts, W. J.: *Uronema elongatum*. New Phytologist, July 1918.



*Uronema indicum* sp. n.

of a filament, about  $28\ \mu$  thick, in which some cells are forming septa, while others are empty.

The alga resembles *Uronema elongatum* very much, but differs from it in its comparatively greater size, in the chloroplast running through the whole length of the cell, and in the cells being generally broader than they are long. For these reasons I propose to give the alga a separate specific name, and term it *Uronema indicum*.

Diagnosing characters of the new alga :

*Uronema indicum*, sp. n. Filaments not enclosed in a mucous sheath, unbranched, straight or a little curved, up to 8 mm. in length. Basal cell attenuated and fixed by a mucous disc. Apical cell acuminate. Other cells cylindrical, generally  $16\text{--}22\ \mu$  thick, but attaining a thickness up to  $40\ \mu$  while forming zoospores. Chromatophore single, parietal, band-shaped, running through the whole length of the cell and passing round two-thirds of the circumference, with incised edges. Pyrenoids up to four in each cell. Nucleus single, median.

Propagation by means of 4-ciliate zoospores, about  $10\ \mu$  in diameter, produced singly from one cell, or in a number from it after its forming transverse and longitudinal septa.

Habit. Found in Lahore, India, sticking to the walls of a dirty drain in the months of March, April, and May, 1919.

## EXPLANATION OF FIGURES.

Figs. 1-2. Basal parts of two filaments, showing the elongated basal cell with the rounded mucous disc.  $\times 500$ .

Fig. 3. A complete filament, showing the beginning of septation in cells.  $\times 500$ .

Fig. 4. A young filament, showing swellings at the joints.  $\times 500$ .

Figs. 5-9. Apical parts of filaments, showing various forms of apical cells.  $\times 500$ .

Fig. 10. Part of a vegetative filament, showing the chloroplast nucleus and pyrenoids in its cells.  $\times 500$ .

Fig. 11. A medium-sized filament, showing the beginning of multiseriate structure, and some empty cells from which zoospores have escaped.  $\times 500$ .

Fig. 12. Part of a filament, showing the escape of zoospores.  $\times 500$ .

Fig. 13. A zoospore, showing four cilia and numerous scattered starch grains.  $\times 800$ .

Fig. 14. Three zoospores germinating together, with confluent basal discs.  $\times 500$ .

Fig. 15. Part of a large filament packed with zoospores.  $\times 600$ .

## Some Anomalies in Monocotyledonous Roots.

BY

AMY VERA SPRATT, B.Sc. Hons. (Lond.).

*University Scholar, King's College, London.*

With Plate III and one Figure in the Text.

AT the end of the last century a good deal of controversial discussion about secondary thickening in *Dracaena* root took place amongst the botanists of the day, and it became fairly established that secondary growth similar to that found in the stem occurs in the larger roots. Caspary in 1858 states that the pericycle becomes meristematic, and this is confirmed by A. de Bary, who states that 'the pericambium assumes the properties and function of extra-fascicular cambium'. In contradiction to these two authorities Strasburger names the cortical cells abutting on the endodermis as the origin of the cambium. Scott and Brebner in 1893 reconciled these two accounts by showing that there are three different conditions of secondary growth: (1) a cambium appears in the pericycle, (2) a cambium appears in the cortex just outside the endodermis, (3) the pericyclic cambium is succeeded by an extra-stelar cambium. These three appear in different regions of the same root.

Although there has been so much discussion with regard to the secondary thickening which takes place outside the stele, very little attention has been paid to the central stele itself. A. de Bary apparently examined roots of terrestrial Aroideae, Pandaneae, and *Dracaena*. His description is somewhat involved, but he says that in the Aroideae the stele consists of a sclerenchymatous cylinder in which wide vessels and sieve tubes are distributed, not lying in radial rows. Pandaneae and *Dracaena*, according to him, exhibit essentially the same structure, except that the sclerenchymatous cylinder is not homogeneous but consists partly of parenchyma. He further adds that in the Pandaneae and *Dracaena* the pith is traversed by sclerenchymatous strands enclosing either vessels or sieve tubes. This, according to A. de Bary, is the primary structure which *Dracaena* roots retain unaltered when secondary thickening as above described occurs. Haberlandt mentions that the root structure of the aerial roots of many epiphytic Aroids, the ordinary roots of certain Palms, and the stilt-roots of *Pandanus* is anomalous. He says that the

central cylinder is dilated and there is a concomitant differentiation of a number of scattered xylem vessels and phloem strands on the inner side of the circle of radially arranged xylem and phloem strands.

The material available in this investigation consisted of the roots of three species of *Dracaena*, namely, *D. fruticosa*, *D. thalioides*, and *D. Draco*; *Cordyline Shepherdii*, *Yucca filamentosa*, *Chamaerops macrocarpa*, stilt roots of *Pandanus*, and aerial roots of *Monstera deliciosa* and *Raphidophora decursiva*. It was fixed in chromacetic acid, and the investigation was carried out by means of hand sections and some microtomed ones stained with methyl green and fuchsin or water safranin and Erlich's haematoxylin.

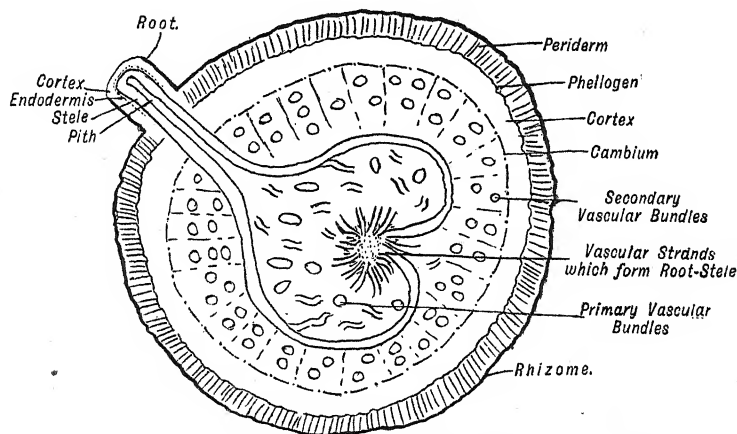
**DRACAENA.** A series of transverse sections was cut through a root of *Dracaena fruticosa* from the tip upwards. In the younger part the sections show the structure of an ordinary Monocotyledonous root, i. e. polyarch. The protoxylems consist of from three to four vessels and the metaxylem vessels are not thoroughly differentiated since the walls are not lignified (see Pl. III, Fig. 1). The vascular elements are surrounded by cells, of which the walls gradually become thickened, so that there is apparently a sclerenchymatous ring surrounding a pith. As the root matures new metaxylem vessels, distinguished by their large diameter, appear in the apparent sclerenchyma, and the phloem also increases somewhat in quantity and breaks up into very numerous small groups, some of which can be seen changing their position from the external to the internal periphery of the band of thick cells. Finally, groups consisting of either a metaxylem vessel surrounded by thick-walled cells, or a group of phloem surrounded by thick-walled cells, are detached from the band into the pith. Frequently these two different groups coalesce in the pith, either one phloem uniting with one xylem or two phloems with one xylem (see Pl. III, Fig. 3). Longitudinal sections revealed that the metaxylem vessels are somewhat peculiar. They have rather an unusual number of transverse walls to be designated vessels, and yet these do not occur so frequently as is customary with tracheides, although the latter may be correlated with the fact that their diameter is larger than that of most tracheides. They have simple multi-seriate pits. The thick-walled cells surrounding these peculiar elements are tracheides of the normal type with simple pits scattered over their walls in irregular fashion (see Pl. III, Fig. 5).

On investigation the root of *Dracaena thalioides* and *D. Draco* were found to exhibit the same peculiar characters as those of *D. fruticosa*.

From the above description it will be evident that a kind of secondary growth takes place in the roots of these three species of *Dracaena*, since new xylem and phloem elements appear in the older roots which do not come from the primary meristems at the growing-points. There are three histogens, one giving rise to the plerome, another to the periblem and



dermatogen, and the third to the calyptragen, which is the normal arrangement in Monocotyledonous roots. Where is the meristem which produces the secondary elements? This point was definitely elucidated by *Cordylina Shepherdii*, which is a near relative of *Dracaena*, a member of the same subdivision of the Liliaceae and greatly resembling *Dracaena* in appearance. The structure of the root is precisely the same as in *Dracaena*, except that in this species, even in the largest roots, the xylem and phloem groups are apparently never budded off into the pith. Apart from this detail there is one difference which became at once apparent, namely, the pericycle is many layered. On examination it is evident that the pericycle in fact acts as a meristematic layer, and here no doubt the new vascular elements originate (see Pl. III, Fig. 2). *Dracaena* being re-examined, it was discovered



Transverse section of rhizome of *Cordylina Shepherdii* with an emerging root. Diagrammatized.

that the pericycle cells, as in *Cordylina*, have contents and they could be seen producing new elements. With this may be correlated the fact that in the *Dracaenas* and in *Cordylina*, in any transverse section, some of the vessel-like tracheides have unthickened walls which undoubtedly thicken as the root matures.

*Cordylina*, as mentioned, is in every way similar to *Dracaena*. With regard to the root, however, there is a distinguishing feature in the fact that *Cordylina* possesses a rhizome (see Pl. III, Fig. 8). This rhizome shows the typical structure of a secondary thickened stem, namely, the central primary vascular bundles and the outer secondary ones. The roots arise endogenously from the rhizome, and when this takes place several of the stem vascular bundles appear to coalesce and form a structure like the primary root stele. At first this stele possesses no endodermis, but this arises when the stele enters the root (see text-figure).

The roots of *Dracaena Draco* become larger than those of the other two species examined. This increase of size no doubt accounts for the fact that this species in the older roots shows a second type of anomalous secondary thickening in addition to that already described. This second type recalls the anomalous thickening of *Dracaena* stem. A cambium is formed just outside the endodermis, and this forms vascular bundles consisting of a few phloem elements surrounded by tracheides (see Pl. III, Fig. 4). The latter are pitted like those in the central root stele, but they are shorter and wider (see Pl. III, Fig. 6).

*Dracaena Draco*, in fact, is one of the species which exhibit the type of secondary thickening similar to that found in the stem and which so many writers have described. The roots at my disposal were quite small, not exceeding half an inch in diameter, and in view of Scott and Brebner's paper it is interesting that in them this secondary thickening was taking place outside the endodermis, not in the pericycle. Previous writers for the most part make no remarks upon the anatomy of the central stele, but according to this research there is another secondary growth which takes place earlier and even in the small roots. Several species of *Dracaena* which do not develop very large roots never undergo this extra-stelar secondary thickening.

*PANDANUS* has been put by A. de Bary with *Dracaena*, as mentioned above. Stilt roots were examined, and in transverse section show a large vascular cylinder with xylem and phloem groups arranged radially round the periphery. The centre has a parenchymatous groundwork, but is largely occupied by groups consisting of pitted tracheides, parenchyma, and phloem, and containing intercellular spaces of large diameter. There are also groups of fibres scattered about; these also appear in the cortex. All the elements found in the stele are differentiated at the growing-point. In transverse section near the tip there are no thickened cells except the scattered groups of fibres mentioned, but otherwise it resembles the stele in the older root. Thus *Pandanus* resembles *Dracaena* in having xylem and phloem elements internal to the vascular cylinder, but shows no secondary thickening. It has an extensive development of the calyptragen, giving rise to a many-layered root-cap which is successively split off in flakes.

*YUCCA FILAMENTOSA* resembles *Pandanus* in that it has internal strands differentiated at the growing-point, but these consist of large and small tracheides as in *Dracaena*. There is no internal phloem. The tracheides possess two rows of pits which, although distinct from those of Gymnosperms, appear to be bordered like they are (see Pl. III, Fig. 13).

*MONSTERA DELICIOSA* (Araceae). The stele of the aerial roots of this plant shows a peripheral ring of xylem and phloem arranged radially. Inside this, it consists of tracheides with simple pits irregularly distributed. Scattered through these there are: (1) xylem vessels with multiseriate

simple oval pits for the most part, but occasionally annular thickening; (2) air-spaces surrounded by slightly elongated parenchymatous cells with contents, i.e. phloem elements (see Pl. III, Fig. 10). In the internal bundles of *Pandanus* the phloem elements surround air-spaces.

Externally there is a slight peculiarity to note about *Monstera*. The young root has a spongy-looking covering, this is followed by a smooth region, and finally we have another spongy one. In the young root there are two very distinct prismatic layers externally. The outer one is slightly lignified and long, blunt, unicellular hairs grow out from it (see Pl. III, Fig. 11). These hairs fail to give any suggestion of blue coloration with either chlor-zinc-iodine or iodine followed by sulphuric acid, but are coloured slightly yellow by an acid solution of aniline sulphate and red by an acid solution of phloroglucin, indicating lignification. In the smooth region the peripheral tissue consists of a cambium which forms thick-walled, brick-shaped cells, suggesting cork, but they very definitely give the lignin reactions quoted above, and on close examination the walls are seen to be pitted. In the older spongy region the same blunt hairs reappear, but below them there are layers of brick-shaped cells with lignified walls as described (see Pl. III, Fig. 12). It seems probable that during the first part of the growing season the root develops hairs, but later ceases to do so, recommencing, however, at the beginning of the next season. As the root becomes older the peculiar lignified cells are developed below the hairs, which at a later period die away, leaving the older part of the root destitute of spongy regions.

These spongy regions recall the velamen of orchids, which likewise gives lignin reactions. In many species of *Pleurothallus* groups of hairs very similar to those in *Monstera* arise from the outermost layer of the velamen. In *Mystacidium infundibulare* they appear to arise when the roots penetrate a substratum, suggesting root-hairs, except that they are lignified. It seems possible that the spongy covering formed by the hairs in *Monstera* root plays a part similar to that of an orchid velamen.

*RAPHIDOPHORA DECURSIVA*. The stele of the aerial root of this Aroid appears fairly normal. The interior of the radial ring is occupied by tracheides pitted as in *Monstera*, and there is also a ring of large vessels placed somewhat irregularly. These have multiseriate, elongated pits. As regards the exterior of the root, *Raphidophora* resembles *Monstera* in having first two definite cell layers and later several produced by a cambium, but the hairs persist throughout (see Pl. III, Figs. 11 and 12).

*Yucca*, *Monstera*, and *Raphidophora* each possess a band of very thick-walled sclerenchymatous cells just outside the stele, which are in each case pitted. In *Yucca* they resemble tracheides in shape; in *Raphidophora* there are some tracheide and some parenchyma-like; and in *Monstera* they are all parenchyma-like.

*CHAMAEROPS MACROCARPA* (Palmae). This resembles *Monstera deliciosa* in transverse section, but it has no phloem except that in the ring. The cells composing the large mass of the stele are tracheides irregularly pitted, and the tracheides of large diameter have multiserial pits, some of which appear to be bordered as in *Yucca*.

The results of this research show that a large proportion of Monocotyledonous Natural Orders contain members which have somewhat abnormal root structures. The anomaly consists in the filling of the pith of the stele, which is rather larger than usual, with scattered vascular elements. These are formed by secondary thickening or are differentiated at the growing-point, and later in some cases coalesce to form a solid stele.

Sincere thanks are due to Mr. Hales, Curator of Chelsea Physic Gardens, for kindly supplying the material, and to Dr. Ethel R. Spratt for supervising the work.

#### SUMMARY.

1. *Dracaena* roots have two distinct kinds of secondary thickening. First, one in which the pericycle becomes meristematic and adds elements to the central stele. In large roots this is followed by a second in which the pericycle, and finally the cortical layers immediately outside the endodermis, are meristematic and form vascular bundles like those formed in the stem.

2. *Pandanus* and *Yucca* have internal vascular strands which are differentiated at the growing-point in the pith.

3. In the aerial roots of some of the Araceae, inside the radial ring of tracheides, vessels and phloem groups are scattered.

4. In all these roots the xylem appears to consist largely of pitted tracheides.

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# EXPLANATION OF PLATE III.

Illustrating Miss A. V. Spratt's paper on Some Anomalies in Monocotyledonous Roots.

*e.* = endodermis; *p.* = pericycle; *ph.* = phloem; *px.* = protoxylem; *t.* = tracheide; *a.* = parenchyma.

Fig. 1. Transverse section of stele of young root of *Dracaena fruticosa*.  $\times 70$ .

Fig. 2. Transverse section of stele of root of *Cordyline Shepherdii*.  $\times 140$ . *y.* = secondary vascular elements.

Fig. 3. Transverse section of stele of older root of *Dracaena fruticosa*.  $\times 70$ . *z.* = group of vascular elements detached from periphery of stele.

Fig. 4. Transverse section of old root of *Dracaena Draco*.  $\times 70$ . *c.* = cortical cambium; *v.b.* = secondary vascular bundles.

Fig. 5. Longitudinal section of central detached group of vascular elements in *Dracaena fruticosa* root.  $\times 325$ . *f.* = fibres; *p.* = pits.

Fig. 6. Longitudinal section of secondary vascular bundle of the root of *Dracaena Draco*.  $\times 325$ . *p.* = pits.

Fig. 7. Transverse section of *Dracaena thalioides* root.  $\times 325$ . *s.* = suberized outer cells; *o.* = cortex.

Fig. 8. Rhizome and root of *Cordyline*. Natural size. *R.* = root; *H.* = rhizome.

Fig. 9. Transverse section of *Pandanus* root.  $\times 70$ . *f.* = fibres; *i.* = intercellular space; *A.* = group of vascular elements.

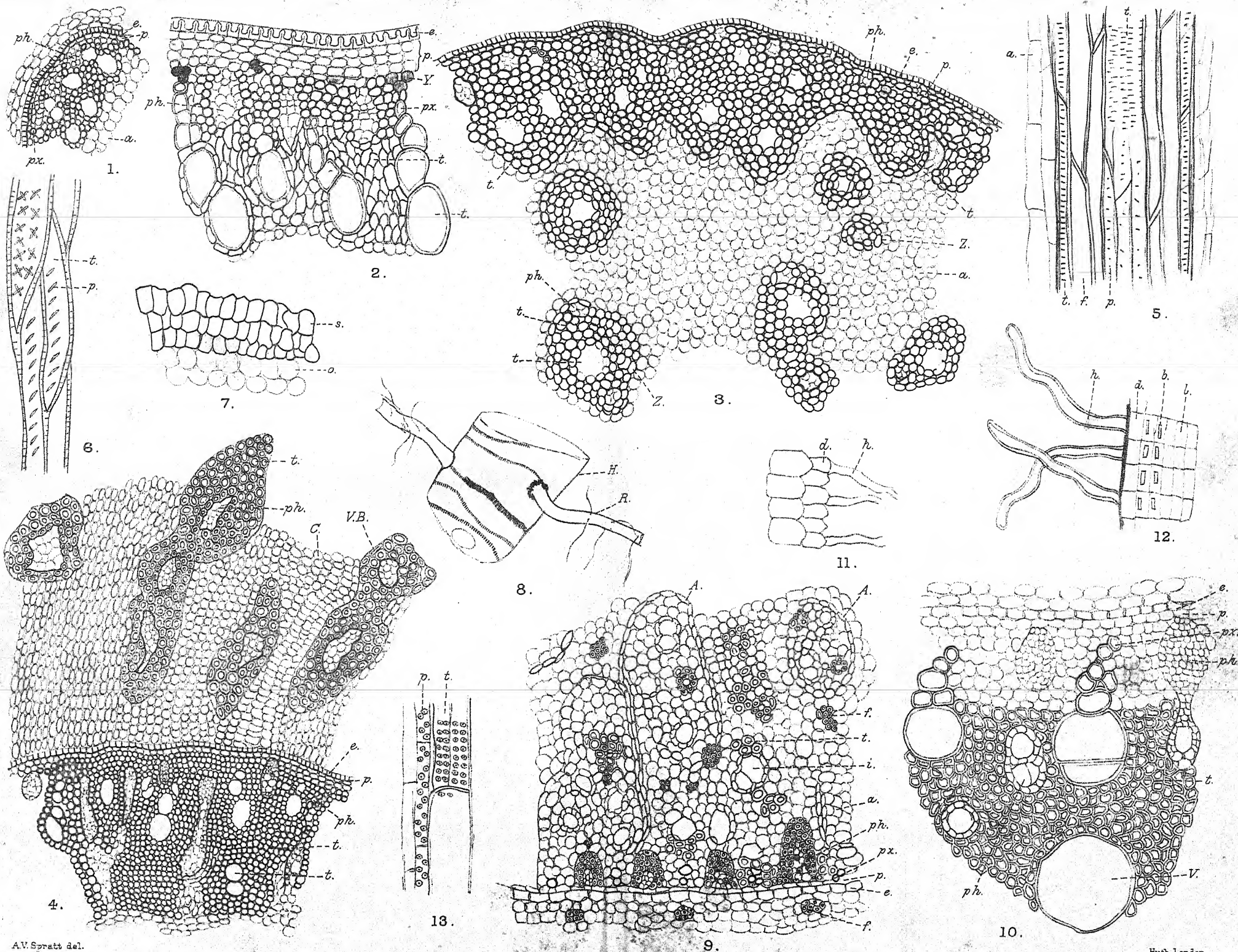
Fig. 10. Transverse section of stele of *Monstera deliciosa* root.  $\times 140$ . *v.* = vessel.

Fig. 11. Transverse section of periphery of young root of *Raphidophora decursiva*.  $\times 325$ . *h.* = hair; *d.* = epidermis.

Fig. 12. Transverse section of periphery of older root of *Raphidophora decursiva*.  $\times 325$ . *h.* = hair; *d.* = epidermis; *l.* = phellogen; *b.* = periderm.

Fig. 13. Longitudinal section of tracheides of *Yucca filamentosa* root.  $\times 325$ . *p.* = pits.





A.V. Spratt del.

Huth, London.

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# Variation in *Anemone apennina*, L., and *Clematis vitalba*, L., with Special Reference to Trimery and Abortion.

BY

E. J. SALISBURY, D.Sc., F.L.S.

With nine Figures and two Tables in the Text.

In the present paper further data are furnished in support of the views already put forward by the author (Salisbury, 1919), relative to the essential trimery of the Ranunculaceae and the variation in number of the constituent parts of the flower through fission or fusion.

## I. *ANEMONE APENNINA*, L.

THIS species affords an interesting subject for comparison with *Anemone nemorosa*, for whereas in that species the prevailing number of perianth segments is six with a range of from 4 to 12 (cf. Yule, 1902; Salisbury, 1919), here the commonest condition is about 16 perianth segments with a range, as exhibited in the 150 flowers dissected, of from 9 to 21.

Of the three regions of the flower the perianth most commonly exhibits departure from the trimerous condition which was found in 34 per cent. of the specimens, a proportion that practically coincides with the mathematical probability.

A noteworthy feature in comparison with its congener is the different form of the variation 'curve'. In Fig. 1 the curves for the two species and *Anemone hepatica* are shown, based on the data of Johnson (1908), Yule, and the writer. It will be seen that the 'curves' for *A. nemorosa* and *A. hepatica* are strikingly similar in general character, whilst that of the former resembles very closely the curve for the perianth of *Eranthis hyemalis* (cf. Salisbury, l.c., Fig. 4, p. 52, 1919). In all three cases the normal number of perianth segments is six, whilst in *A. apennina*, where the normal number is much higher, the mode is near the centre of the curve. In other words, whilst in *A. apennina* the tendency towards diminution is as great as that of multiplication where the normal perianth number is low, the tendency is almost entirely in the direction of increase.

That this association is a real one is indicated by the change in form of the variation curve for the perianth of *Ranunculus bulbosus* which de Vries attained (1893) by repeated selection. Curves based on de Vries'



figures (cf. Fig. 2) show an asymmetrical form where the average of the strain was five petals but an almost symmetrical form in the selected strain where the average number was nine.

In the one hundred and fifty flowers whose parts were carefully dissected, the androecium consisted of from 48 to 111 stamens, both limits, as those for the perianth, being multiples of three. The most frequent

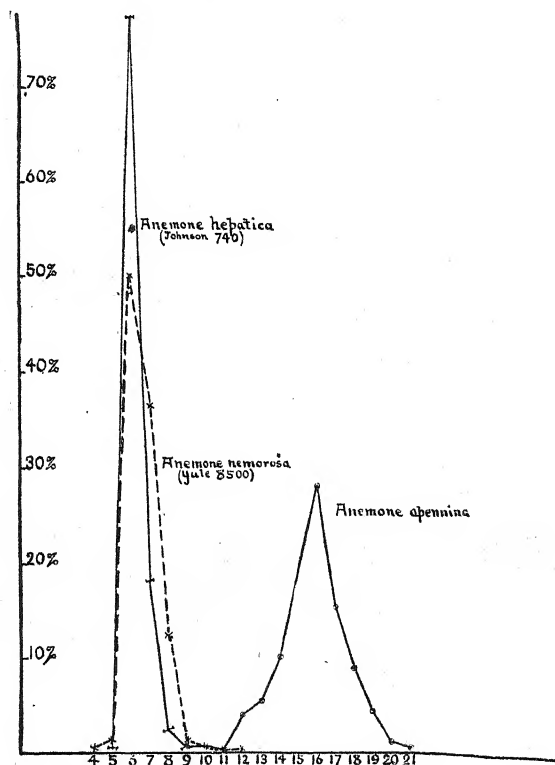


FIG. 1.

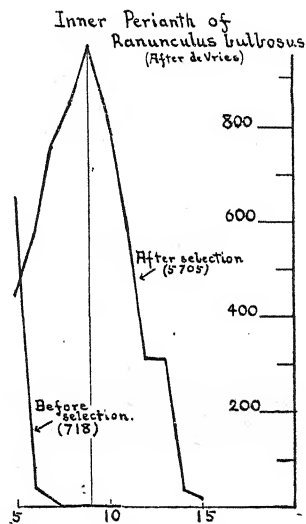


FIG. 2.

conditions as shown by the 'curve' (Fig. 3) were 72, 81, and 87. The marked periodicity of the 'curve' is obviously related to a succession of trimeric modes. In no less than 55.3 per cent. of the flowers examined the staminal number was some multiple of three.

Departure from the modes can here again be related to fission or fusion. Four flowers in which two anthers were present on a common filament exhibited a number of stamens which was a multiple of three (63, 63, 75, and 78) if the branching were ignored.

The essential trimery of the gynaecium was even more pronounced, since the number of carpels was a multiple of three in 57.3 per cent. of the flowers, the most common conditions being 60, 57, and 63 carpels respectively.

Not only does the variation 'curve' show the same periodicity with modes corresponding to multiples of three, but the decreasing prominence of these as we pass away from the primary mode is clearly exhibited (cf. Fig. 4). The range is from 27 to 87, so that both in the gynaeceum and androecium the numbers compared with *Ficaria verna* (androecium 15-63 and gynaeceum 8-55) are high. It may therefore be of significance that the 'curves' in that species are very asymmetrical, whilst here they are very much less so. Comparison within the same genus is, however, of greater value, and in the two species of *Anemone* (*A. nemorosa* and *A. apennina*) where

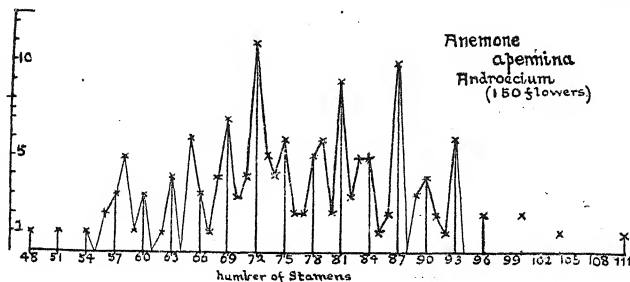


FIG. 3.

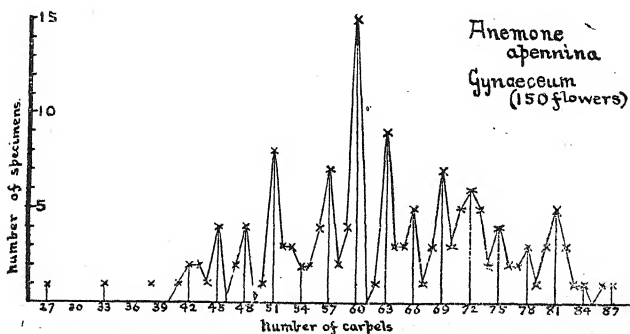


FIG. 4.

the average carpel number is so different the 'curves' show a marked contrast in respect to the degree of asymmetry.

One instance of a carpel bearing two stigmas was observed, again suggesting fission as the cause of departure from the trimorous condition.

The large number of perianth segments in this species renders it particularly suited to a study of their origin. Five flowers exhibited petaloid stamens in which the anthers were represented to a more or less marked extent. Just as we cannot distinguish the stamens which have undergone complete fission, so too here we cannot recognize the completely petaloid stamens, since the latter do not in this genus bear any nectary. There is no doubt that both methods of origin contribute to produce

variation of the perianth, but there are good reasons for regarding congenital fission, of the original segment rudiments, as the chief cause. The arrangement of the supernumerary parts is one ground for this view, but much more significant is the relation which the perianth number bears to the stamen number. From the following table it will be seen that the average number of stamens associated with a given number of perianth segments increases instead of decreasing when the latter is large.

<i>Number of Perianth Segments.</i>	<i>Average Number of Stamens.</i>
9	54
10	51
11	50
12	63
13	68
14	72
15	72
16	77
17	82
18	82
19	86
20	96
21	90

That a negative correlation obtains between perianth number and stamen number when the former is augmented by transformation of the latter has been shown in the case of *Chelidonium majus* by Karl Sax (1918), and conversely Bonnier noted (1889) that petal-less flowers of *Atragene alpina* always had more numerous stamens than normal petalled flowers.

It is of course patent from the work of Goebel (1905) and others (e. g. Saunders, 1917) that doubleness in its extreme form may involve transformation of stamens, which even in the same species (cf. Saunders, 1917) may or may not be accompanied by multiplication of parts. It is nevertheless important to guard our minds against the assumption that doubleness, in the popular use of the term, signifies a homogeneous group of phenomena, and still more, from the unsupported conclusion that the normal polymeric perianth is a phenomena of precisely the same nature as that exhibited in the extreme 'double' flowers of horticulture. In reference to the non-homogeneity of the phenomenon of doubleness it may be pointed out that in two closely allied species of *Dianthus* Miss Saunders' has shown that 'doubleness' is dominant in the one and recessive in the other—a fact difficult to reconcile with the presence and absence hypothesis except on the assumption that the 'doubleness' is of a different character in the two cases. We may also note that in the somewhat parallel phenomenon of fasciation White (1917) considers this morphological feature to be due to very varied causes (p. 490).

## II. CLEMATIS VITALBA, L.

The meristic variation in the gynaecium of *Clematis vitalba* was investigated in 1,202 specimens. As shown in Fig. 5, the curve exhibits a strikingly periodic form with maxima at multiples of three as in most of the genera and species already studied. But here the feature is more emphasized and the progressive decrease of the secondary modes is also well illustrated.

The importance of the secondary modes, and even the position of the primary mode, though always corresponding to a multiple of three, appear to vary with the individual and even in different parts of one and the same plant. Thus the specimens studied were obtained from three localities in the neighbourhood of Radlett. Those from the different localities gave very similar variation curves, but whilst from the one, gynaecia consisting of twenty-seven carpels predominated, from the other two localities, gynaecia with twenty-four carpels were commonest. It is unlikely that this difference was due to any variation in the habitat conditions, which were apparently almost identical; moreover, similar differences were noted when comparison was made of the different plants in the same locality.

Just as different primary modes may characterize the various species of a genus, so too their relative prominence may vary with the individual. An interesting point, however, and one which is important in this connexion, is how far the individual is

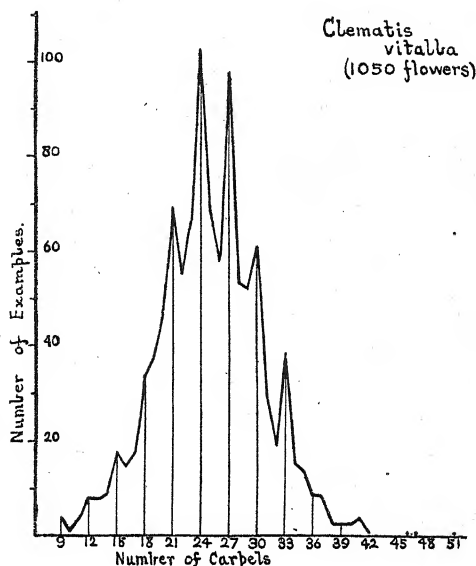


FIG. 5.

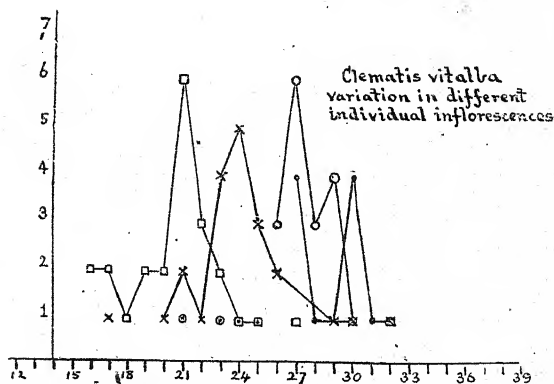


FIG. 6.

homogeneous in this respect. The data obtained from several hundred inflorescences were compared from this point of view, and the marked differences which those from the same branch may exhibit, both as regards variation range and the position of the mode, is illustrated by the curves for four separate inflorescences of the same plant reproduced in Fig. 6.

The marked trimery exhibited by the gynaeceum of this species is all the more interesting since the genus is characterized by a relatively primitive carpel in the sense that the functionless rudimentary ovules are retained (cf. Warming, 1913). One may hazard the suggestion that the marked trimery is associated with this gynaeceal conservatism. In respect

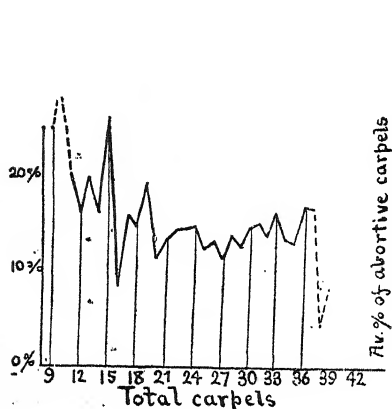


FIG. 7.

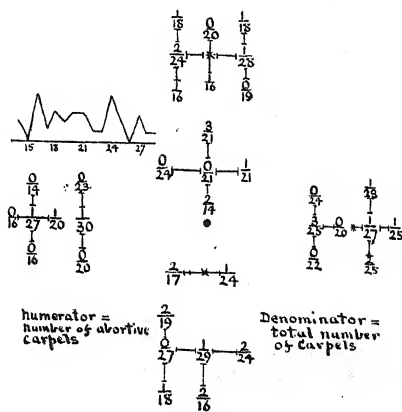


FIG. 8.

to this trimery the individual inflorescence bears much the same relation to the plant as a whole as does the 'pure line' to the homozygous strain from which it is selected. This tendency towards a segregation within the soma of the individual may be responsible for the fact that if one selects seed from flowers with a high number of perianth segments the offspring exhibits a higher average than do those raised from seeds produced in flowers of the same plant with a low perianth number. In Fig. 8 is shown a diagrammatic representation of a branch bearing a number of small inflorescences, and by its sides the variation curve for the gynaecea. It will be apparent that the range is relatively narrow, and in the different flowers of the individual inflorescence the total number of carpels exhibit a striking similarity. Here too, as indeed is generally the case, the terminal flower of a dichasium usually contains a greater number of carpels than the lateral flowers corresponding to it. Their relation is probably largely dependent on conditions of nutrition, the first-formed flower being naturally favoured in this respect. Exceptions are nevertheless by no means infrequent, and, especially in crowded inflorescences, the gynaeceum of the peripheral lateral

flower may exhibit a numerical superiority not only over that of the corresponding interiorly placed lateral flower but also over that of the terminal member of its cyme.

Examples of branched carpels were found in eighteen fruit heads, or about 1.5 per cent. Of course this represents under 1 per cent. of the total number of carpels examined, but in view of the fact that only where the process of fission is incomplete is the phenomenon recognizable we may conclude that such is probably by no means infrequent and may account for the variation about the different modes.

ABORTIVE CARPELS IN *Clematis vitalba*. CORRELATION BETWEEN FERTILE AND ABORTIVE CARPELS.

Total No. of Carpels.	Number of Abortive Carpels.																			Totals.
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
9	1	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	4
10	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
11	1	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
12	3	2	1	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	8
13	1	2	1	1	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	8
14	1	-	5	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	9
15	1	3	2	2	2	3	1	3	1	-	-	-	-	-	-	-	-	-	-	18
16	4	6	2	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	15
17	3	4	4	1	1	1	2	2	-	-	-	-	-	-	-	-	-	-	-	18
18	5	12	6	2	3	-	-	1	2	2	1	-	-	-	-	-	-	-	-	34
19	4	5	5	7	6	4	1	2	1	-	-	1	1	1	-	-	-	-	-	38
20	11	6	10	6	9	2	-	2	1	-	-	-	-	-	-	-	-	-	-	47
21	6	18	14	11	7	6	3	1	1	1	1	1	-	-	-	-	-	-	-	70
22	7	8	13	8	6	4	2	3	1	3	-	-	-	-	-	-	-	-	-	55
23	5	9	15	11	7	3	9	1	2	2	1	1	-	-	-	-	-	-	1	67
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31	1	7	2	10	-	-	1	2	1	2	2	-	-	1	1	1	-	-	-	31
32	1	3	4	2	1	1	-	2	2	-	3	-	-	-	-	-	-	-	-	19
33	1	4	4	5	4	4	4	1	2	3	2	2	1	-	-	-	1	-	-	38
34	2	1	3	1	4	1	-	1	-	-	-	1	1	-	-	1	-	-	-	16
35	-	2	-	3	2	1	1	2	1	1	-	-	-	-	-	-	-	-	-	13
36	-	-	2	1	1	1	1	-	-	1	1	-	-	-	-	1	-	-	-	9
37	-	-	1	2	2	-	-	1	-	1	1	-	-	-	-	1	-	-	-	9
38	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
39	-	1	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	3
40	-	-	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	3
41	-	-	1	-	1	1	-	1	-	-	-	-	-	-	-	-	-	-	-	4
42	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
46	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
47	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
51	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Totals	115	177	179	159	113	70	54	42	37	28	19	12	13	7	3	5	1	2	0	1037

Abortion.

Abortive achenes are of such frequent occurrence in *Clematis vitalba* that a relatively small proportion of the seed heads consist entirely of fertile fruits. The abortive achenes can be recognized by the fact that the body of

the carpel is relatively flat in contrast with the robust swollen character of those which are fertile. Two types can, however, be recognized. In one the ovary remains quite small and the length of the style is only about 4 to 5 mm. These doubtless represent carpels which have not been pollinated. It is significant of the effectiveness of the entomophilous pollination of this species that only an insignificant proportion of this type was encountered. The second type, representing about 98–99 per cent. of the abortive fruits, embraces those in which the ovary has undergone enlargement, though the absence of endosperm results in the flattened character already referred to. In these the style is almost, if not quite, as long as in fertile achenes. In the study of abortion in this plant we are not therefore dealing with the idiosyncrasies of a pollinating agent, but with some more subtle factor.

The number of abortive carpels was determined in 1,040 of the gynaecea examined, and a glance at the correlation table on p. 113 shows that there is a definite tendency for the number of abortive carpels to increase with the increase in total number of carpels. The proportion nevertheless diminishes, as shown by the slight downward trend of the percentage curve (cf. Fig. 7).

As the more numerous carpels are generally associated with the better nourished flowers, the extent of abortion would seem to be also determined by conditions of nutrition and favourable development. In order to obtain evidence on this point the average percentage of abortive carpels in a number of terminal fruiting heads was compared with that of the corresponding laterals. The value of the former was found to be 11.7 per cent. and of the latter 13.6 per cent. That is to say, the proportion of carpels which abort is greater in the lateral flowers than in the terminal.

*Additional Note on Ficaria verna.*

Since the publication of the writer's previous paper in which the variations of this species were dealt with, several further papers on this species have come to his notice. Of these, the most important are those by F. Ludwig (1908), Prof. Macleod (1907), and A. A. Dallman (1915 and 1916). In the first of these a number of tables are furnished which show the correlation between sepals and petals in *Ficaria verna*. The corresponding correlation coefficients were subsequently worked out by Miss Lee (1908) for the various localities from which the specimens were obtained and show a positive correlation in most cases which seems to preclude the acceptance of the transformation theory ( $0.1928 \pm 0.0205$ ;  $0.1954 \pm 0.0203$ ;  $0.0188 \pm 0.0260$ ; and  $0.2237 \pm 0.0203$ ). In the second paper Prof. Macleod gives correlation data for the number of stamens and pistils of 373 late flowers and 268 early flowers. From the figures there furnished the curves illustrated (Fig. 9) were constructed, and it will be noted that the androecia of the late flowers show two modes corresponding to fifteen and eighteen stamens respectively,

whilst the early flowers exhibit a prominent mode at twenty-seven. In the 'curve' for the gynaecia of the early flowers no trimery is apparent, but the more numerous examples of late flowers show a clear tendency in this direction. Prof. Macleod's figures, therefore, though emphasizing the trimerous tendency, do not show the periodic grouping to so marked an extent as the specimens examined by the writer.

It may perhaps be well to emphasize the fact that in all the specimens dealt with in this investigation the parts have invariably been counted twice to avoid possible error, and wherever necessary examined under the dissecting microscope to ensure the inclusion, in the total, of aborted rudiments and the recognition of any precocious abscission. To avoid error from the latter cause such examination is particularly necessary where unopened buds are not exclusively employed.

In the last-named paper Mr. Dallman has collected data regarding the perianth of *Ficaria verna* from over 49,000 flowers derived from a number of widely separated localities. The results show that though the mode is the same in all cases the relative proportion of specimens showing increase or decrease may vary with the locality or season. The

range in sepal number observed was from 0-6 and of petals from 4-19. The latter figures are identical with those given by Babington (1834). In reference to the calyx Mr. Dallman expresses the doubt as to whether true asepalia occurs, and, indeed, in all the examples studied by the writer, in which there were apparently less than three sepals, this was found to be due to precocious abscission or petaloidy of one or more members. With respect to the corolla the upper limit in the present writer's specimens, viz. eleven, appears to be seldom exceeded, and the three examples cited by Mr. Dallman with seventeen, eighteen, and nineteen petals respectively are admitted to have been the outcome of fasciation.

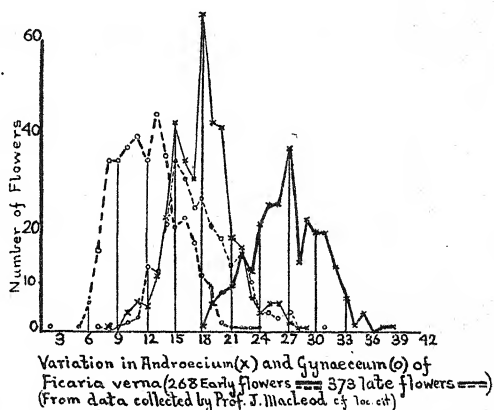


FIG. 9.



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# On Certain Archaic Features in the Seed of *Taxus baccata*, with Remarks on the Antiquity of the Taxineae.

BY

B. SAHNI, M.A., M.Sc.

*Emmanuel College, Cambridge ; Professor of Botany, Benares.*

With seven Figures in the Text.

## INTRODUCTORY.

THE examination of *Taxus* seeds on which this paper is based was originally undertaken in connexion with an investigation of the New Caledonian genus *Acmopyle*, an account of which it is hoped to publish in the near future. In that paper the conclusion is expressed that the general *Taxus*, *Torreya*, and *Cephalotaxus* are structurally so distinct from the Podocarps and other Conifers, that they deserve the rank of a separate phylum, Taxales,<sup>1</sup> having a clearer and more direct relation with *Ginkgo* and the Cordaitales than may be claimed for the remaining plants generally grouped under the Conifers. Since the following observations—which have a strong bearing upon the Cordaitean theory of the ancestry of Conifers—would have unduly lengthened my paper on *Acmopyle*, they are here published separately.

Professor Oliver's work (1903) has clearly shown that *Torreya* possesses one of the most primitively organized seeds known among living Gymnosperms. From his study of the seed of *Cephalotaxus* Worsdell (1900) has come to a similar conclusion with regard to this genus. These considerations, and the close similarity in general morphology between the ovuliferous shoots of *Cordaitea* and *Taxus*, impelled me to make a search for possible archaic features in the seed of *Taxus*, and to compare the latter with what we know of the structure of Cordaitean seeds.<sup>2</sup>

As a result of this comparison I have been led to conclude that, so far as seed-structure is concerned, (1) *Taxus* is in some respects more

<sup>1</sup> A name previously adopted by Lotsy (1911, p. 160), but not in the sense of a group equal in rank with the Coniferales.

<sup>2</sup> The previous investigations on the seed of this common plant, numerous as they are, have chiefly been conducted from the point of view of the structure and development of the nucellus, gametophytes, and embryo, while the minute study of the integument has been confined, so far as I know, to the comparatively young stages.

primitively organized than *Torreya*, and (2) the Taxales are in their affinities nearer to *Ginkgo* and to the Cordaitales than to any other known plants.<sup>1</sup> The general similarity which the seeds of *Torreya* and *Cephalotaxus* show to that of *Ginkgo* was recognized long ago (Lindley, 1836, p. 317; 1853, p. 231) by an association of these three genera in the same order (Taxaceae of Lindley), till the discovery of motile sperms in *Ginkgo* led to a separation of this genus as the sole survivor of an ancient phylum. The Ginkgoales are now universally regarded as being related on the one side to the Cycads, and on the other to the Cordaitales; especially to the latter group, of which they may claim to be more or less direct descendants. On the other hand, the retention of *Torreya* and *Cephalotaxus* in the position long ago assigned to them by Lindley (in the order Taxaceae, including such genera as *Dacrydium* and *Podocarpus*) has tended to emphasize their affinity with the Conifers. I venture to think that this emphasis has led to a false impression as to the affinities of the Yews, which, as already suggested, appear to lie rather with the Cordaitales and with *Ginkgo* than with the Podocarps. A further discussion of this question with special reference to the position of *Phyllocladus* will be given in my forthcoming paper on *Acropyle*, but it may be stated here that the view that there is no close relation between the Yews and the Podocarps has previously been expressed by several authors, notably in a recent article by Dr. Scott (1911).

#### DESCRIPTIVE.

*Ovule.* The structure of the young ovule of *Taxus* is too well known to need further description. But the fact is worth recording, that in my microtome series of longitudinal sections (in the principal plane) a distinct vascular supply comes off from the two main supply bundles, and enters the base of the aril (Fig. 1). The aril supply is, however, so inconspicuous that it is not surprising that it escaped the notice of the earlier workers (Van Tieghem, 1869, p. 281; Strasburger, 1872, p. 4), who had only hand-sections at their disposal.

*Young Seed.* Owing to the hardness of the integument<sup>2</sup> the oldest stage of which coherent microtome sections were obtainable is that shown in Fig. 2. In this case the aril supply was completely obscured by the mass of very dark-staining tissue in that region. In the slightly earlier stage shown in Fig. 3 the supply to the aril is seen to consist of a row of scalariform tracheides; phloem could not be identified with certainty, so that the orientation of the aril supply is doubtful. In the figure the tracheides are shown in solid black, while the dotted region represents thin-walled elongated cells, possibly phloem.

<sup>1</sup> See also Lotsy (1911, p. 5), where a similar view has been expressed.

<sup>2</sup> It was necessary to keep the sliced seeds in melted paraffin (60° C. melting-point) for two to three months before sectioning.

After removal of the aril the bicarinate seed of *Taxus* shows a small elliptical disc slightly sunk into the base of the seed, and pierced by two<sup>1</sup> minute foramina in the principal plane (Fig. 4, *a*). The longitudinal section shows that the disc consists of several layers of palisade-like simply pitted cells (Fig. 5, *a*) different from the thicker-walled and isodiametric stone-cells composing the rest of the shell (Fig. 5, *b*).

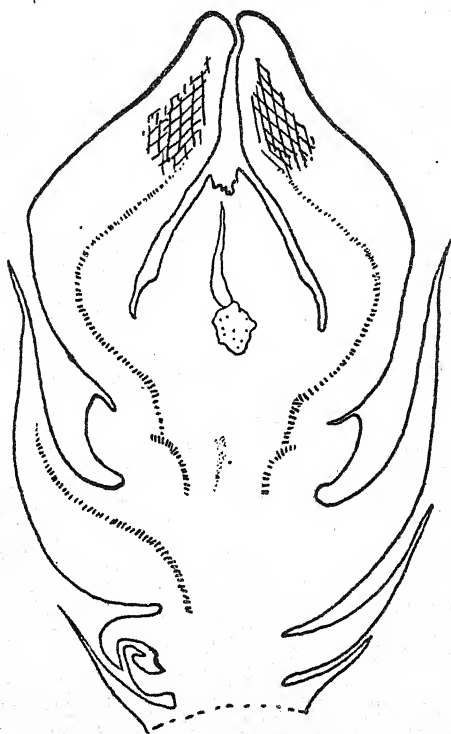


FIG. 1.

The foramina in the basal disc are the external openings of two straight canals which convey the two supply bundles of the seed obliquely upwards into the seed-cavity. In its further course towards the micropyle each strand steadily approaches the inner face of the shell, until at a short distance from the micropyle it actually comes into contact with the latter and runs along its inner surface for some little distance before dying out (compare the young stage in Fig. 1).

Special mention should be made of the fact that immediately after entering the seed-cavity each of the two supply bundles always shows

<sup>1</sup> Seeds with two or more planes of symmetry have a corresponding number of foramina (see Fig. 4, *b*, *c*).

a slight but easily noticeable thickening beyond which, instead of proceeding in the continuation of the canal, it bends sharply outwards so as to form a rather striking knee-like bend. The presence of these thickened angles in the bundles is a point on which I wish to lay stress, in spite of its apparent insignificance, for I have reason to believe that they mark the

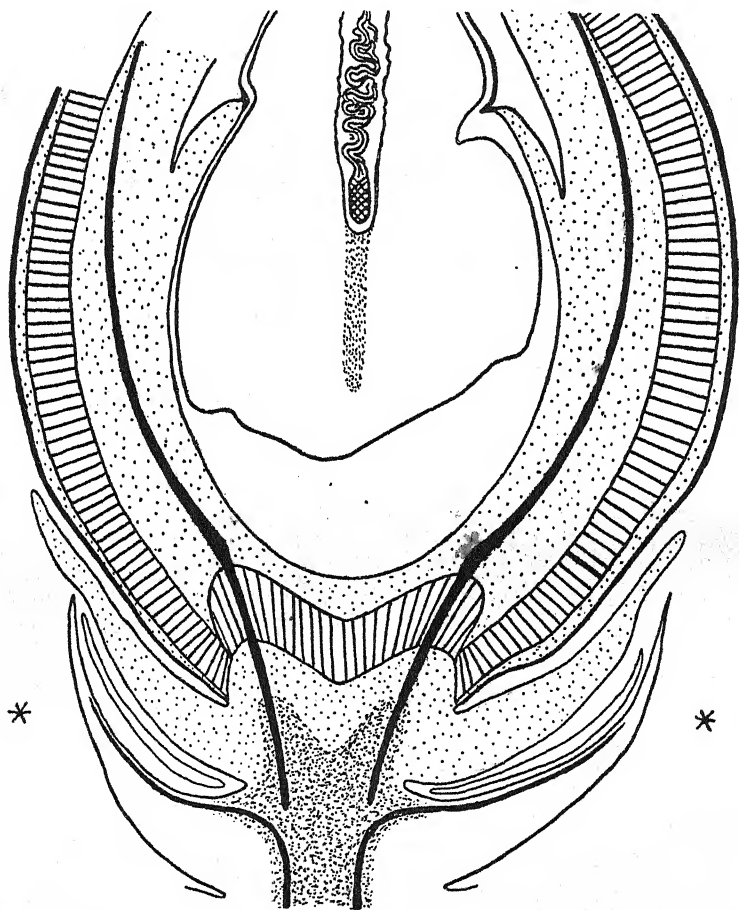


FIG. 2.

points from which once branched off an 'internal' (nucellar) system of strands, now extinct in *Taxus*.<sup>1</sup>

That I am able here to state my reason for this view, I owe to Prof. R. H. Compton, M.A., who very generously allowed me to examine the ovules of an extremely interesting new genus of Conifers recently

<sup>1</sup> It is indeed not impossible that if a sufficiently advanced stage of the seed could be sectioned—a process beset with practical difficulties owing to the bony sclerotesta—actual vestiges of an 'internal' vascular system might be discovered.

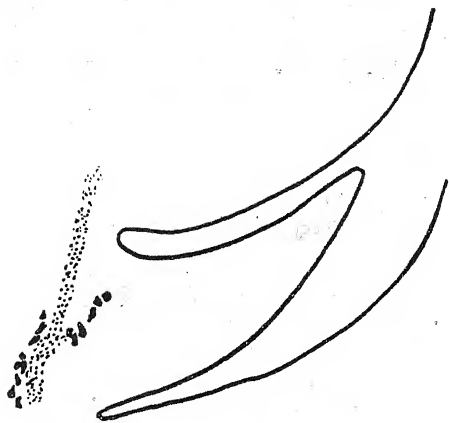
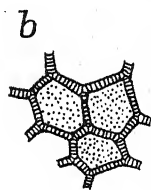


FIG. 3.

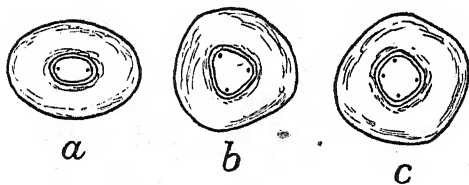


a



b

FIG. 5.



a

b

c

FIG. 4.

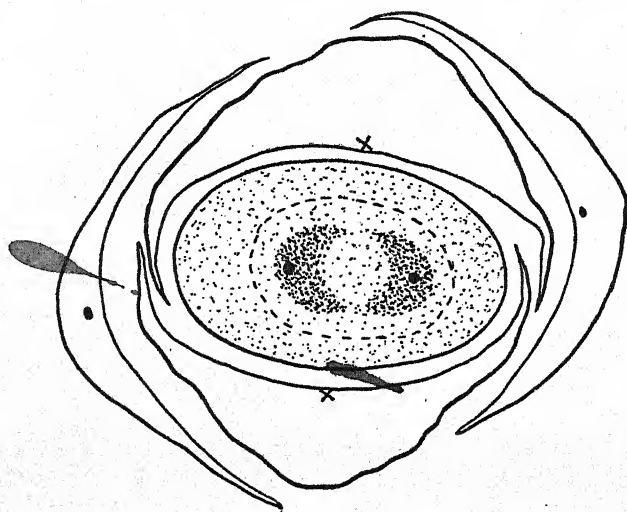


FIG. 6.

discovered by him in New Caledonia. So far as the female organs are concerned, this plant might at first be taken for a large-sized species of *Taxus*, the ovule being terminal on a bracteate axillary shoot, and the seed having a well-developed but free cup-shaped aril; but a noteworthy difference is that there is a distinct 'internal' (nucellar) vascular system. A fact of further significance from our point of view is that the nucellar strands branch off from the main bundles at points roughly corresponding to those in *Taxus*, which are here also marked by slightly thickened knee-like bends.

Attention may be drawn to the close proximity of the nucellus to the thickened knee-like bends in the strands of *Taxus* (Fig. 2). This part of the vascular supply is in fact nearer to the nucellus than is any other part.

Fig. 6 from a transverse section at the level \*\* in Fig. 2 is given to show the striking similarity to Sprecher's figure of *Ginkgo* (1907, p. 134, Fig. 143); the broken line shows the region along which, at a higher level, the aril separates off as a ring. The vascular bundles of the bracts are very close to the adaxial surface: in the inner pair of bracts they are extinct at this level, their positions being marked by crosses.

#### THEORETICAL.

In 1903 Professor Oliver, in his well-known paper on 'The Ovules of the Older Gymnosperms', showed how a seed like that of *Torreya* may be derived from a type such as the Palaeozoic *Cardiocarpus*, with a free nucellus and a centralized main vascular supply at the base, entering the seed-cavity through a single median foramen in the stone. He postulated longitudinal splitting of the main supply of the ancestral seed-type into the two strands which now pass into the seed-cavity through two foramina lying in the principal plane. At the same time it is supposed that during the ancestral history of *Torreya* the base of the seed underwent a marked transverse expansion,<sup>1</sup> with the result that these two foramina, while retaining their position in the principal plane, gradually receded from each other; and through the widening base of the seed the nucellus and megaspore bulged downwards into the apex of the seminiferous axis. This phylogenetically younger portion of the seed, conveniently termed by Professor Oliver the hyposperm, is in *Torreya* so large in proportion to the archisperm (defining the original limits of the seed) that the 'ancestral chalaza' is actually nearer to the micropyle than to the lower end of the entire seed.

<sup>1</sup> This transverse expansion of the seed-base must be distinguished from the longitudinal extension postulated by Professor Oliver in deriving the seed of *Cycas* from the same ancestral type. Both processes result in the intercalation of a new region at the base, but the main supply splits longitudinally in the one case, while in the other case it remains unaffected. In this paper we shall concern ourselves only with the former case.



If this has really been the course of events in the history of *Torreya*, the seed of this genus must belong to a truly isolated type, and would seem to be the result of specialization along a restricted side line. Nevertheless, the fact that the three known genera of *Taxales* so naturally fall into line with each other in most other respects justifies the expectation that it should be possible also to make the peculiar seed-organization of *Torreya* intelligible in terms of *Taxus* and *Cephalotaxus*. An attempt to do this is described in the following pages, which at the same time appear to corroborate in some detail the ingenious theory advanced by Professor Oliver sixteen years ago.

The two foramina in the basal disc of the sclerotesta of *Taxus* naturally recall those well known to occur in *Torreya* at a much higher level. Since it is tolerably certain that the paired ovular strands in both genera have been derived by a splitting of an original central main supply, it would seem that *Taxus* is nearer to the ancestral type in so far that in the former genus the 'chalazal foramina' are nearer the middle line than in the latter.

But in such a comparison between *Taxus* and *Torreya* there is one apparently insuperable difficulty, namely the fact that whereas in *Torreya* the strands of the 'outer system' are entirely outside the stone, in *Taxus* they are entirely inside the seed-cavity, for, as is well known, in this genus the outer flesh (if this name is at all applicable) is represented by only a thin membrane of un lignified cells covering the stone.

How, then, is it possible to derive these two apparently divergent types from the same ancestral form? ✓

We will, in the first instance, fix our attention upon certain well-preserved platyspermic seeds of Cordaitan affinity, described long ago by Ad. Brongniart (1874) from the Carboniferous of St. Étienne, and subsequently (1881) refigured to show the details of their structure. The original diagnoses have to some extent been recently modified by Professor C. E. Bertrand (1907, 1, 2, 3, 4, 1908, 1, 2, 3), who undertook a revision of Brongniart's types with the help of Renault's later preparations. More recently Mrs. Agnes Arber (1910) has made an interesting contribution to our knowledge of these ancient seeds, which exhibit a remarkable diversity of form without departing from the essentials of the plan upon which they are built.<sup>1</sup>

<sup>1</sup> The further literature is referred to in Mrs. Arber's paper. The attribution of the seeds in question is, with the exception of *Cardiocarpus*, still a matter of conjecture, but there is a strong presumption in favour of the view that like *Cardiocarpus* they all belonged to members of the phylum Cordaitales. Apart from their agreeing with *Cardiocarpus* in their general plan of structure, they all possess a more or less well developed 'tent-pole'—an organ which appears to be of considerable importance in questions of relationship. In the case of one of these seeds (*Mitrospermum*) Dr. Scott has recently (1918-1919) discovered some indirect evidence to show that it was probably borne upon the highly specialized axillary shoots of the Cordaitan species *Mesoxylon multirame*.



On a general survey of this assemblage it was noticed that if a certain well-marked tendency (which I shall presently explain) was kept in view, it was possible to arrange the seeds in a series, as follows:

*Cardiocarpus*; <sup>1</sup> *Cycadinocarpus*; *Rhabdospermum*; <sup>2</sup> *Mitrospermum*; *Taxospermum*.

Of course, it is not by any means suggested that this series represents the actual course of evolution, but it appears as if the tendency it expresses may have been a real factor in the history of these ancient seeds, which, as already remarked, are all approximately of the same age. And this view at least acquires some interest when it is noticed that, so far as this tendency is concerned, the series appears to be continued into the living genera *Torreya* and *Cephalotaxus* in one direction, and into *Taxus* in another. See Fig. 7, Nos. 2-II.

A word of explanation is necessary in connexion with the figures referred to. All the figures are purely diagrammatic, but with two exceptions they do not represent any essential facts not already published. These exceptions are:

(a) The dotted lines in *Mitrospermum* (No. 6) represent the 'inner' (nucellar) vascular system, and have been inserted on the presumption that their absence in Mrs. Arber's material was due to imperfect preservation.<sup>3</sup> The resemblance of *Mitrospermum* to *Rhabdospermum* (in which the 'inner' strands are clearly seen in Brongniart's figures) is far-reaching enough to justify this step.

(b) In *Taxospermum* (Fig. 7), too, which is likewise fundamentally similar to *Mitrospermum*, traces of an 'inner' system have been observed by C. E. Bertrand (1907, p. 216) at a level higher than the inner openings of the obliquely ascending lateral canals in the shell. The continuation downwards of these strands is thus not entirely unjustified, although their exact points of insertion must remain doubtful till better-preserved material is available.

It will be seen that whereas in the first member of the series (*Cardiocarpus*, Fig. 2) the 'outer' system of strands is entirely outside the stone, as we pass along the series its points of origin from the main supply

<sup>1</sup> As defined by Professor C. E. Bertrand (1908).

<sup>2</sup> A generic name proposed by Professor Seward (1917, p. 341) for seeds of the *Rhabdocarpus* type showing anatomical structure, Berger's original name *Rhabdocarpus* being reserved for impressions.

<sup>3</sup> Since writing the above I have, through the great kindness of Dr. D. H. Scott, F.R.S., been able to examine some well-preserved sections of *Mitrospermum compressum*; although these sections did not show any sign of an internal vascular system, it may be stated that none of them happened to pass quite through the right plane. The question as to the existence of internal bundles in this seed is therefore open. It is quite possible that no internal bundles existed in *Mitrospermum*, which in this respect would hold the same relation to *Rhabdospermum* as, among living genera, *Taxus* does to the new Taxinean genus recently discovered in New Caledonia by Prof. Compton (*vide supra*).

gradually shifts nearer and nearer to the subnucellar pad of tracheides which also gives rise to the 'inner' system. During this process the basal parts of the two outer strands may be imagined to cut their way through the stone, somewhat like a hot piece of wire stretched tight and pressed against a block of wax, so that the regions through which it has passed have

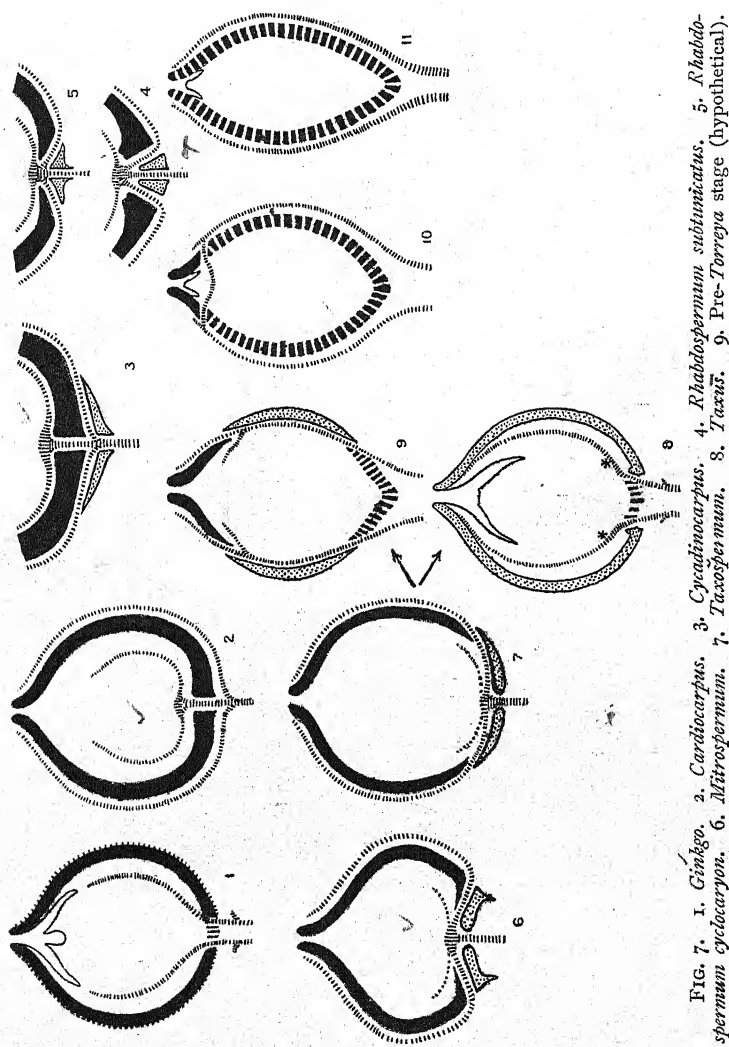


FIG. 7. 1. *Ginkgo*. 2. *Cardiocarpus*. 3. *Cycadiocarpus*. 4. *Rhabdospermum subunicatus*. 5. *Rhabdospermum cyclocaryon*. 6. *Mitrospermum*. 7. *Taxospermum*. 8. *Taxus*. 9. Pre-*Torreya* stage (hypothetical). 10. *Torreya*. 11. *Cephalotaxus*.

again solidified. In the figures these regions of the stone have for the sake of clearness been distinguished by a different method of shading. Thus we see that the proximal parts of these strands, ascending and entirely outside the stone in *Cardiocarpus*, become horizontal and partly included in the

stone in *Cycadinocarpus*, while in *Rhabdospermum* they actually turn obliquely backwards and outwards to emerge from the stone through two foramina not far from the point where the main supply enters the stone. For convenience I propose to describe as excurrent and incurrent canals, respectively, those channels in the sclerotesta which afford egress or ingress to the vascular bundles.

Now, if we imagine that the outer openings of the excurrent canals of *Rhabdospermum* gradually move forward towards the micropyle, the conditions observed in *Mitrospermum* and *Taxospermum* will successively be arrived at: the canals will first become nearly horizontal as in *Mitrospermum* and then ascending as in *Taxospermum*. For our purpose the most important result of this tendency is that more and more of the 'outer' system of strands becomes included inside the shell-cavity. Thus in *Taxospermum* a considerable length of the 'outer' system is really inside the stone.

At this point I shall venture a suggestion which at first perhaps appears unwarranted, but which, as I hope to show presently, is not entirely without a foundation. Assuming that this acropetal tendency on the part of the excurrent canals did not become extinct in Palaeozoic times, is it not possible that the modern genus *Taxus* (in which the entire length of the 'outer' system of strands is inside the seed-cavity) may represent the culmination of this tendency? In other words, may it not be that in this plant the excurrent canals have actually reached the micropyle and become confluent with it? If this is so, it follows that that part of the stone which in *Taxospermum* lies distally to the excurrent canals, (shown solid black in Fig. 7, No. 7) is entirely unrepresented in *Taxus*; and, further, that the proximal part of the stone of *Taxospermum* (shown dotted) corresponds to almost the whole of the shell of *Taxus*. The single incurrent canal of *Taxospermum* having split into the two incurrent canals of *Taxus*, that part of the stone of *Taxus* lying between these canals must be regarded as a newly intercalated piece.

Direct comparisons of this nature between plants so widely separated in time do not often lead to safe conclusions. But the suggestion here put forward is at any rate the only one that affords at all a satisfactory interpretation of the peculiar seed-structure of *Taxus*; and, as we shall see, it also makes it possible to compare the latter intelligibly with *Torreya*. When we realize the magnitude of the time-gap between *Taxospermum* and *Taxus* in the light of this suggestion, the structural gap between them becomes surprisingly small; and the vague comparison which Ad. Brongniart instituted between the two seeds as long ago as 1874, and which he expressed in the generic name *Taxospermum*, now acquires a phylogenetic significance. I wish to avoid conveying the impression that I regard *Taxospermum* as being necessarily a direct ancestor of the modern Yew,

for we are yet wholly ignorant as to the nature of the plant that bore the Palaeozoic seed, but the resemblances between the seeds certainly appear to be due to more than a chance. Such points of agreement as those in general shape and size may be ignored as being superficial. But it is at least a fact of some significance that, so far as known, of all the fossil seeds which show evidence of a Cordaitan affinity, it is *Taxospermum* that shows the greatest amount of fusion between nucellus and integument: an important advance in the direction of *Torreya* and *Taxus*. (See Bertrand, 1907, p. 216.)

Apart from the difference in the position of the 'outer' vascular system—which I have just attempted to explain—the most important difference between the two seeds is that in *Taxus* there are two basal supply bundles instead of the single one in *Taxospermum*. But there is every justification for the view that the two basal ovular strands, lying in the principal plane, which are a striking characteristic of the Ginkgoales, Taxales, and Coniferales, have been derived by the splitting of a single median bundle; for it is an observed fact that a centralized main supply prevails in the overwhelming majority of known Palaeozoic Gymnospermous seeds, whether platyspermic or radiospermic. If we imagine the two incurrent canals of *Taxus* to approach each other and fuse into a single median canal, the two contained bundles at the same time fusing into one, the thickened angles in these bundles would coalesce into a vascular mass exactly corresponding in position to the subnucellar tracheal pad characteristic of most Palaeozoic seeds with a centralized main supply. It is now easy to see the significance of the fact, to which attention was drawn in the descriptive part of the paper, that these thickened angles in the ovular strands of *Taxus* are situated nearer to the nucellus than any other part of the vascular supply.

The absence of a definite 'internal' (nucellar) system of strands in *Taxus* will hardly be taken as a serious objection to the comparison here instituted, for it is doubtless related to the absence of fertilization by motile sperms. I have already (p. 120) offered some reason for the view that the thickened angles in the ovular strands in *Taxus* probably mark the points of origin of the nucellar bundles in the ancestral type, although these bundles have become extinct in the modern genus.

Having considered whatever evidence there is for deriving the seed of *Taxus* from a type like *Taxospermum*, we shall now attempt to see in what relation the seeds of *Torreya* and *Cephalotaxus* stand towards either of the former seeds. Nos. 7-11 on Fig. 7 illustrate the view which I consider to be the most plausible. It will be noticed that I have attempted to derive the seed of *Torreya* also from the same source (*Taxospermum*) but along a line distinct from that of *Taxus*. At first sight the seed of *Torreya* appears so different from *Taxospermum* that it is difficult to see how a

comparison is possible. But if we agree with the conclusion reached by Professor Oliver, that the part of the seed lying below the level of the 'chalazal foramina' is a phylogenetically recent formation, the difference from *Taxospermum* does not appear so great. For the sake of clearness, I have interposed between these two seeds a hypothetical intermediate condition (Fig. 7, No. 9) which may for convenience be referred to as the pre-*Torreya* stage. Between *Taxospermum* and pre-*Torreya* there are only two points of difference. Firstly, pre-*Torreya* has at the base two strands which enter the stone through two incurrent canals, and between which an appreciable portion of the sclerotesta has been newly intercalated, just as in *Taxus*. Secondly, the excurrent canals in pre-*Torreya* are nearer to the micropyle than they are in *Taxospermum*—it has already been suggested that they have a tendency to move towards the micropyle. The dotted portion of the stone in Fig. 7, No. 9, may thus be described as having grown at the expense of the part shaded with lines, to the extent that the excurrent canals have moved forward. At the same time, however, the newly intercalated portion of the stone (shown cross-hatched) must be regarded as having increased in size at the expense of the dotted portion of the stone, for, ever since the splitting of the main supply bundle of the ancestral type, the two incurrent canals (conveying the resulting bundles) have also steadily travelled towards the micropyle.

Pre-*Torreya* is thus a strange kind of seed, in which the stone is pierced in the principal plane by two pairs of canals (one excurrent, the other incurrent), both of which are, in a phylogenetic sense, moving towards the micropyle. From this stage *Torreya* is only one step farther. We have only to imagine that the hinder pair of canals, having moved faster than the front pair, has overtaken the latter and become confluent with it. A glance at the figures will show that the obvious result of this process is that the dotted region of the stone is gradually squeezed out of existence. It will thus be seen that the incurrent canals of *Taxus* are not strictly comparable to those which convey the nucellar bundles of *Torreya* into the seed-cavity—a want of correspondence which is in keeping with the fact that the strands conveyed by the canals in the two genera are themselves not homologous, being the main supply bundles in the case of *Taxus* and nucellar bundles in *Torreya*.

It is of some interest to find that although we have derived the seed of *Torreya* from the *Cardiocarpus* type through a long succession of varying forms, our interpretation of this peculiar seed entirely agrees with that which Professor Oliver arrived at from a direct comparison between *Torreya* and *Cardiocarpus*. If we look back at the series of figures from *Torreya* to *Cardiocarpus*, it will be seen, from the conventional method of shading that we have throughout adopted, that in *Torreya* the small apical region of the stone is the representative of the entire stone of the ancestral type, which

has steadily undergone reduction, having been encroached upon in succession by two phylogenetically younger portions of the sclerotesta, intercalated one after the other at the base of the seed. Each of these newly formed portions of the stone has shown a persistent tendency to grow in the direction of the micropyle. The modern genus *Taxus* illustrates the triumph of the older of these, which forms almost the whole of the sclerotesta in this genus; in *Torreya* it is the younger of these newly created regions of the stone that protects nearly the whole of the seed.

Turning now to the third genus, *Cephalotaxus*, the question arises in what light we are to regard the stone of this seed. We have seen that in the ancestral history of *Torreya* the 'chalazal foramina' have probably shown a continual tendency to shift nearer and nearer to the micropyle. In *Cephalotaxus*, however, the most careful search reveals no trace of any perforations in the stone. May we conclude that in this genus the 'chalazal foramina' have actually passed beyond the micropyle—that the entire stone of *Cephalotaxus* corresponds to the newly intercalated portion of the stone of *Torreya* and to the small basal disc of *Taxus*? This, at any rate, seems to me the most natural conclusion, strange as the suggestion may appear. Of course, the alternative suggestion may be made that the seed of *Cephalotaxus* differs essentially from that of *Torreya* only in having lost its nucellar bundles by reduction *in situ*, and that consequently the 'chalazal foramina' which once admitted these strands have also become obliterated. If it were shown that the young seed contains vestiges of the nucellar bundles, or of foramina in the stone which become blocked up as development proceeds, this view would be indisputable. But in the absence of positive evidence on either side the conjecture first put forward is preferable, being more in conformity with the general line of argument previously adopted. The fact that there are in *Cephalotaxus*, as in *Torreya*, two separate supply bundles at the base would alone suggest that the part of the stone lying between them is a comparatively recent formation; and since the bundles run up as far as the micropyle without branching, this conclusion must evidently apply to the entire stone.

If the tendencies outlined above have had any significance in the evolution of the seeds in question, we have further evidence for the view—which is already well established on other grounds—that the Yews are descended from Cordaitalean ancestors. For the fact that it is possible to arrange these seeds into a more or less continuous series, helps us further to bridge the structural gap between the two groups, wide though the gap in time may be. The hope may therefore be well founded that rocks of an intermediate age will reveal evidence of the existence of seeds transitional between *Taxospermum* on the one hand, and *Torreya* and *Taxus* on the other.

The comparison of the seeds of the Taxales with the older seeds



appears, however, to fail in this respect, namely, that, so far as is known, the 'aril' was entirely unrepresented in the Cordaitales. But it is difficult to estimate the value of negative evidence, for, apart from the question of preservation, these fossil seeds are nearly always found detached, and the possibility is not excluded that if an aril was present it was left behind on the seminiferous axis when the seed was shed. At least in one of the few known cases where the attachment is preserved—namely, *Cordaianthus Williamsoni*—Renault's well-known figure (see Scott, 1909, p. 540) shows below the seed a differentiated pad of tissue terminating the seminiferous axis. May we regard this pad of tissue as the forerunner of an aril? Since I have not seen the original specimen this suggestion must be regarded as of the most tentative nature; but if the aril of *Taxus* is in origin a disc-like expansion of the apex of the seminiferous axis—and this view, held by Strasburger in 1872, is perhaps more plausible than any other<sup>1</sup>—it is natural to expect that in such an ancient type as *Cardiocarpus* the aril would not be well differentiated from the rest of the axis. It would seem as if this disc, as it gradually became differentiated into a distinct organ, was in time completely appropriated by the seed, and was shed along with it as in *Taxus*.

Strasburger long ago (1879, p. 124) expressed the view that the seeds of Gymnosperms are often more or less deeply sunk into the seminiferous axis, so that it is no longer possible to define their real limits. This view found a remarkable illustration when Professor Oliver described the main features in the development of the seed of *Torreya*, and published his ingenious interpretation of this peculiar type of seed. In this genus that part of the seed which lies below the level of the 'chalazal foramina' in the stone is, according to Professor Oliver, phylogenetically younger than the apical region, which (in a figurative sense) may be described as having bulged downwards, through the widened seed-base, into a kind of 'brood-pouch' formed by what is strictly speaking the apex of the seminiferous axis. This expanded apex of the axis has, however, been so completely monopolized by the encroaching seed that it is now, to all appearance, an integral part of the integument; distally it is continued into the free part of the aril, which has had a similar origin at an earlier period, as suggested above in the case of *Taxus*.

If we keep in view this tendency on the part of the seed to bury itself deeper and deeper into the seminiferous axis, the seed of *Cephalotaxus* would appear to have gone even farther than that of *Torreya*, for the free portion of the nucellus which belongs to the archisperm (or phylogenetically older part of the seed) is in *Cephalotaxus* actually arched over by an

<sup>1</sup> Since *Ginkgo* is traceable to the same source as the Taxales, it is probable that the 'collar' of this genus is morphologically of the same origin as the 'aril' of *Taxus*. Sprecher (1907) actually employs the term 'aril' in describing the collar of *Ginkgo*.

integument which, as already suggested, is a comparatively recent formation.

In attempting to assign a place to *Ginkgo* in relation to the Cordaitales ✓ the fact must be taken into account that there is apparently nothing in the seed of this plant to represent the 'outer' vascular system. There may, however, be some significance in the fact, observed by Sprecher (1907, p. 137), that the superficial cells of the young sclerotesta of *Ginkgo* are histologically indistinguishable from tracheides (see Sprecher's Fig. 150, p. 137). May the outer vascular system of *Ginkgo* be regarded as having fused up with the outer surface of the stone? The following remark by Sprecher is of some interest in this connexion: 'Le noyau scléreux me paraît être en rapport avec le tissu de transfusion de l'arille; il est sa continuation dans l'arille.' If this view is justified *Ginkgo* would appear to make a remarkably near approach to the Palaeozoic seed *Cardiocarpus*, in which the 'outer' system of strands comes off from the main supply before the latter enters the stone (cf. Nos. 1 and 2 in Fig. 7). This would corroborate the low position of *Ginkgo* indicated by the motile sperms and the well-developed pollen-chamber and 'internal' vascular system, as well as by the presence of a well-developed 'tent-pole'. Another fact pointing in the same direction is the proximity of the two supply bundles to the middle line, which shows that the main supply has, in a phylogenetic sense, only recently undergone splitting.

#### SUMMARY AND CONCLUSIONS.

The present paper contains an elaboration of a theory developed by Professor Oliver in 1903, when he offered an interpretation of the seed-structure of *Torreya* in terms of the Palaeozoic seed *Cardiocarpus*, which was regarded as the ancestral type. It is suggested that the Palaeozoic seeds *Cycadinocarpus*, *Rhabdospermum*, *Mitrospermum*, and *Taxospermum*, all of which probably belong to the Cordaitan phylum, illustrate the general tendency that may have operated in producing the types of seed known to occur in the modern genera *Taxus*, *Torreya*, and *Cephalotaxus*. It is proposed to place these three genera in a distinct phylum, Taxales: the members of this phylum are regarded as the nearest existing relatives—apart from *Ginkgo*—of the Cordaitales, and like *Ginkgo* direct descendants of the Cordaitales. While accepting a Cordaitalean origin for the Coniferales, I regard the further question—whether the connexion was a direct one or whether the Conifers arose as a branch from the Taxales—as still *sub judice*, although I am inclined to favour a direct connexion. The problem as to the ultimate microphyllous or megaphyllous origin of the Cordaitales (and therefore also of the Conifers) is left untouched in this paper.

If the seeds of the Taxales have been derived from those of the



Cordaitales along the general tendencies outlined above, it is clear that we cannot strictly speak of a homology between the respective layers of the integument (outer flesh, stone, and inner flesh). A glance at the figure will show, for example, that in the so-called stone of *Taxus* there is nothing really comparable with the stone of *Cardiocarpus*. The region of the integument of *Taxus* which is comparable to the stone of the Palaeozoic type is no longer lignified and has become part of the inner flesh. It lies along a plane just inside the 'outer' vascular system, and would be represented by two lines joining the marks \* \* with the micropyle. In *Taxus*, therefore, what we are accustomed to call the 'stone' is really homologous with a portion of the 'outer flesh' of the ancestral type.

In comparing the seeds of the Taxales among themselves and with those of the Cordaitales the positions of the two vascular supply systems may safely be relied upon as determining the real homologies of the different parts of the integument.

I wish to express my gratitude to Professor Seward for the kind interest he has shown in this paper by his able advice and criticism. To Mr. R. L. Lynch, M.A., Curator of the Cambridge University Botanic Garden, I am indebted for some of the material of *Taxus baccata* used in this investigation. I would also like to record my hearty thanks to Dr. Scott and to Mrs. Agnes Arber for valuable criticisms. To Dr. Scott I am further deeply indebted for his very generously lending me some of his best sections of *Mitrospermum*. Lastly, I have also gratefully to acknowledge Professor Seward's kindness in reading the proofs of this paper.

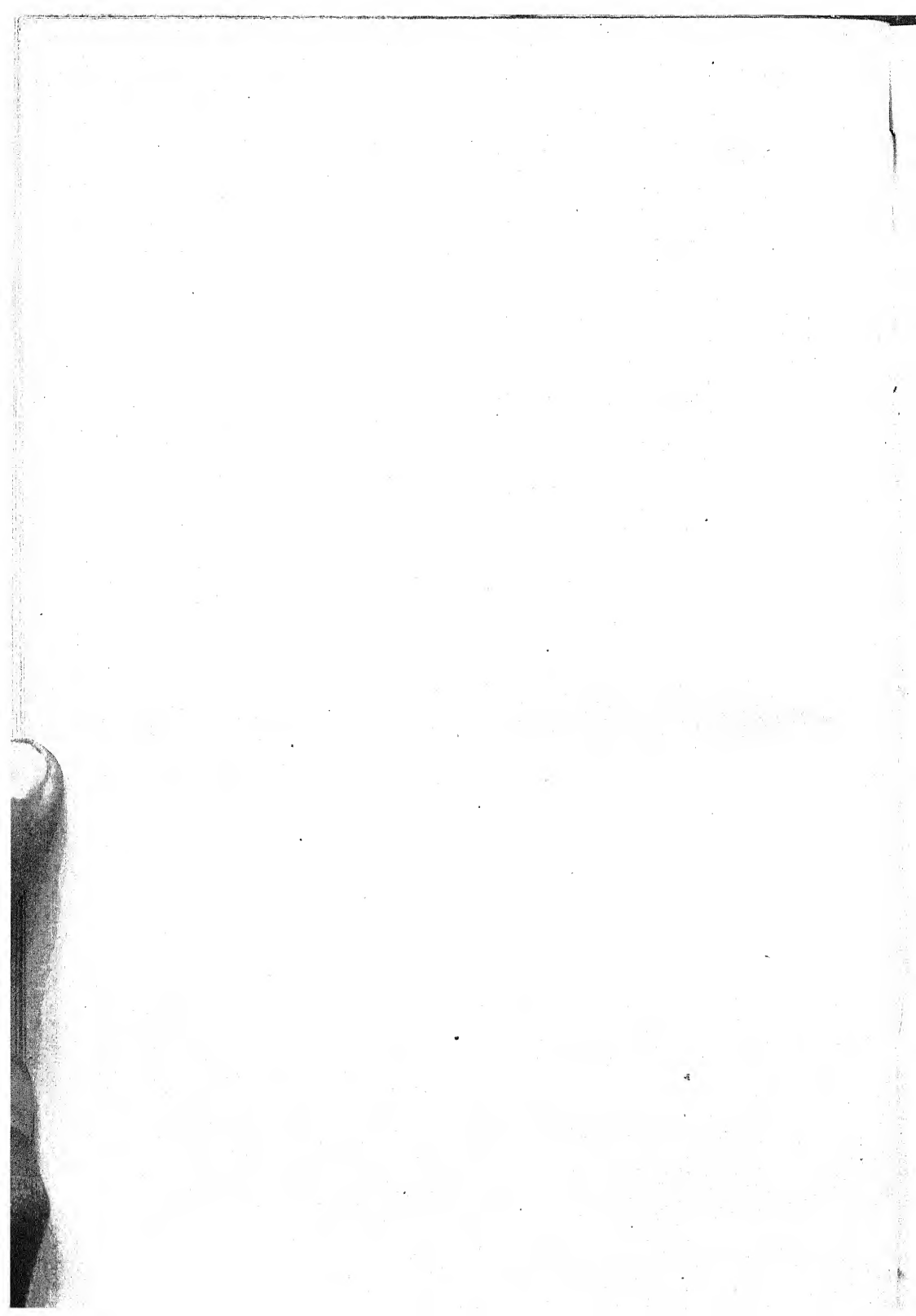
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April 7, 1919.

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## NOTES.

CANTHELIOPHORUS, BASSLER: NEW RECORDS OF SIGILLARIO-STROBUS (MAZOCARPON).—In my recent paper<sup>1</sup> I regret having overlooked some interesting incrustation specimens which Nathorst had recorded<sup>2</sup> some years previously from the Palaeozoic rocks of Spitzbergen. After figuring them and discussing their nature he concludes as follows:

‘Als Resultat unserer Untersuchungen kann nur gesagt werden, dass dieselben wahrscheinlich die Mikrosporophylle eines bisher unbekannten *Lepidostrobus*- oder *Lepidocarpus*-Typus darstellen, dessen Sporangien durch ihren komplizierten, vorläufig aber nicht näher zu bestimmenden Bau von den bisher bekannten *Lepidophyllum*-Arten abweichen.’

In the August number<sup>3</sup> of the ‘Botanical Gazette’ of 1919, Bassler brought the above specimens into line with a number of American incrustation fossils not hitherto described. I owe to him, therefore, the fact that my attention has been drawn to this large number of new specimens. Bassler has, however, misinterpreted, as I conceive, both Nathorst’s specimens and his own. He thinks they all exhibit ‘a large lamellar sporangiophore developed in the radial plane of the strobilus from the superior face of the sporophyll pedicel, bearing two large elongate sporangia, one upon each side, pannier-like’; and it is this interpretation which has suggested the generic name *Cantheliophorus* (κανθήλια, pack-saddle with panniers) for both Nathorst’s specimens and his own.

Thus Bassler thinks he has discovered in these impressions evidence of ‘a truly sporangiophoric Lepidophyte’ and thus shown that the Lepidodendreae ‘are not the homogeneous, stereotyped group they were long supposed to be’.

Before proceeding to discuss his position I will state at once that all the species included in Bassler’s new genus appear to me to admit of an alternative explanation.

It is unfortunate that Bassler, owing possibly to his work being carried out in a geological laboratory, has overlooked the full account of *Mazocarpus*, or I feel sure he would have realized the features of resemblance to the microsporophylls there described. He only refers to a very inadequate preliminary reference to *Mazocarpus*<sup>4</sup> which was published before the critical specimens had been obtained. In my view the bulk of the specimens figured, if not all, belong to various species of *Sigillariostrobus* (*Mazocarpus*) and represent microsporophylls which have become separated from the cone axis.

<sup>1</sup> Benson: *Mazocarpus*, or the Structure of *Sigillariostrobus*. Ann. Bot., vol. xxxii, 1918, p. 569.

<sup>2</sup> Nathorst: Zur fossilen Flora der Polarländer. Nachträge zur palaeozoischen Flora Spitzbergens, Teil I, 1914, p. 62.

<sup>3</sup> Bassler: A Sporangiophoric Lepidophyte from the Carboniferous. Bot. Gaz., vol. lxxviii, 1919, p. 73.

<sup>4</sup> Benson: The Sporangiophore. New Phyt., vol. vii, pp. 143-9, 1908.

To make my position clearer I will refer to a few of the figures in detail :

*Lepidophyllum mirabile* as shown in Nathorst's photomicrograph (Nathorst, loc. cit., Taf. 13, Fig. 27) should be compared with Text-fig. 4 B in the *Mazocarpon* paper. Though less bulky, the resemblances in the form of the sporange and of the bract are unmistakable. Nathorst states : 'die Partie über dem oberen Feld scheint in einer dreieckigen Spitze oberhalb der Blattlamina frei zu endigen'. If we also note that the sporophylls are detached from their cone axis, we see that we have several characters strongly indicative of the *Sigillariostrobus* microsporophyll. The trabeculate character of the sterile tissue and the suggestion of a longitudinal ridge or lamina are also in harmony with this interpretation. If the *Mazocarpon* paper had antedated Nathorst's 'Palaeozoic Flora of Spitzbergen', Teil I, it is probable that he would himself have included these specimens in *Sigillariostrobus*.

Turning now to Bassler's specimens we find he recognized nine species, of which seven are new to science. Specimens of each are figured. All are found, like Nathorst's, to be detached from the cone axis. When found in their original relative position to one another the axis has perished—a condition similar to that of the microsporophylls of *Sigillariostrobus* recorded by Kidston.<sup>1</sup> They all show the characteristic prolongation of the sporange beyond its line of insertion on the bract. Indications of the *Mazocarpon* position of the vascular bundle in the pedicel and not in the keel are possibly seen in Bassler's Fig. 22, and several specimens (Figs. 1-3, 16 and 27) suggest the occurrence of a so-called 'lateral lamella' which is characteristic of *Mazocarpon* and possibly is the true interpretation of the line referred to as 'the brace' (Bassler, loc. cit., p. 79), while the region Bassler calls 'the crest' has been called in *Mazocarpon* 'the ridge'.

It seems unnecessary further to discuss the details, as a comparison of the figures can hardly fail to convince the observer that we are dealing in both with the same type of structure.

For convenience I will tabulate what I regard as the more important indications that most, if not all, of Bassler's new specimens can be interpreted as *Sigillariostrobus*.

1. Their general occurrence free from the axis of the cone.
2. The form of the sporange and of the bract.
3. The occurrence of lateral lines, some of which suggest the vascular pedicel and some the 'lateral lamella' of *Mazocarpon*.
4. The indication of a bulky sporange wall and the relatively small spore-bearing region.

With respect to Bassler's grouping of his specimens into numerous species, I should like to state that I do not consider the data are sufficient in every case. There were sporophylls on a single cone in *Mazocarpon* which showed as wide a range as that between *C. linearifolius* (Lx) and *C. grandis*.

Some of the specimens (Bassler's Figs. 4 and 19-21) which have been reproduced from Nathorst's work representing *Lepidophyllum riparium* and *L. waldenburgense* I should prefer to leave as Nathorst left them, as nothing is to be gained by attempting to interpret them further than as sporophylls bearing sporangia 'of some-

<sup>1</sup> Kidston : On the Fossil Flora of the Yorkshire Coal Field (second paper). Trans. Roy. Soc. Ed., vol. xxxix, Part I, 1897, Pl. II, Fig. 1.

what complex structure'. They are perhaps as much like *Lepidocarpon* as *Mazocarpon*.

I trust I have adduced sufficient evidence to show that there is no adequate ground for assuming the existence of a sporangiophoric Lepidophyte. On the other hand, most of the remarkable specimens now for the first time collected and figured by Bassler are welcomed as further examples of Sigillarian microsporophylls, of which we had hitherto only one incrustation record (Kidston, loc. cit., Pl. II, 1, esp. d').

M. BENSON.

ROYAL HOLLOWAY COLLEGE,  
UNIVERSITY OF LONDON.

**ON THE GEMMAE OF *TORTULA MUTICA*, LINDB.** Among mosses, the production of gemmae is a comparatively rare phenomenon, as is seen from the fact that of the six hundred and twenty odd British species adopted by Braithwaite<sup>1</sup> only seventeen are known to reproduce themselves in this manner.

In these seventeen species the form and origin of the gemmae are exceedingly various. They may be red, club-shaped, septate processes which become detached from the margins of the leaf; or they may grow in clusters at the leaf-apex. More complicated gemmae are sometimes met with in the axils of the leaves, and these are often red and may develop directly into bulbils which become detached from the parent plant. In the most highly specialized plants the gemmae are borne within special cups of leaves, or on leafless pseudopodia.

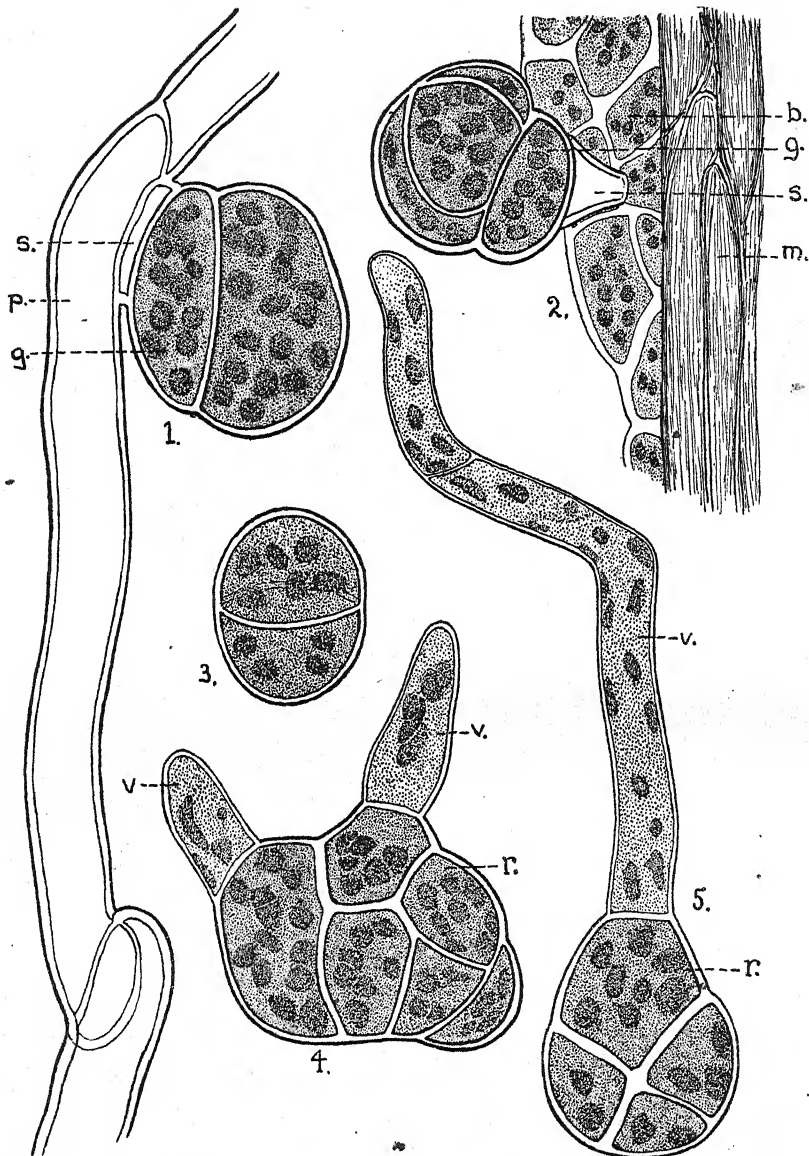
Braithwaite records that in one specimen of *Tortula mutica* which he had examined there were 'minute globular gonidia scattered over the surface of the leaf, not unlike those in *Tortula papillosa*'.<sup>2</sup> His record is extremely brief, and no figure is given.

Recently, however, a specimen of *Tortula mutica* from North Wales has been examined, and was observed to bear numerous gemmae. They were simple in form and were usually borne scattered over the surface of the leaf. Each consisted, as a rule, of two (Fig. 3) or four (Figs. 2 and 5) cells bounded by thick, reddish-brown cell-walls, and containing dense granular protoplasm and a number of large, circular or somewhat irregular, discoid chloroplasts. Occasionally larger gemmae were found (Fig. 4), and in these the disposition of the cells was more irregular than in the smaller gemmae. The gemmae were attached to the leaf of the mother-plant by means of a single colourless stalk-cell, which grew from the surface of one of the cells of the blade, as shown in Fig. 2. The chloroplasts in the gemmae were very much larger than those in the cells of the leaf-blade, while their colour was a deep bluish-green, far more intense than that observed in the leaf itself.

Much more rarely, gemmae were found growing laterally on protonema-filaments whose walls had assumed a brown colour almost as dark as that of the cell-walls of the gemmae, and whose contents were destitute of chloroplasts. These gemmae were attached to the protonema-filament by means of a short stalk-cell, and rarely consisted of more than two green cells (Fig. 1).

<sup>1</sup> R. Braithwaite: British Moss Flora. London, 1887.

<sup>2</sup> Loc. cit., i, p. 222.



FIGS 1-5. Gemmae of *Tortula mutica*, Lindb.  $\times 825$ . 1. 2-celled gemma growing on protonema-filament; 2. 4-celled gemma attached to leaf-blade; 3. Small gemma detached from leaf; 4. Larger gemma beginning to germinate in two places; 5. 4-celled gemma with single young protonema-filament. b., Cell of leaf-blade; g., gemma; m., midrib of leaf; p., protonema-filament; r., resting cell of gemma; s., stalk-cell; v., young protonema-filament.



The gemmae easily became detached from the parent plant by the complete breaking down of the stalk-cell, and then appeared to enter upon a period of rest. They germinated, after a shorter or longer interval, by the outgrowth of a protonema-filament from one of the resting cells. These young filaments had a thin colourless wall, and contained a relatively small number of pale green chloroplasts that were somewhat elongated in the direction of the long axis of the cell.

In the germination of the smaller gemmae a single protonema-filament only was produced (Fig. 5), but in the larger ones sometimes more than one of the resting cells resumed activity, as is shown in Fig. 4, where two young outgrowths are depicted.

These gemmae of *Tortula mutica* differ from those figured by Braithwaite<sup>1</sup> for *T. papillosa* chiefly in their position and mode of attachment. In *T. papillosa*, gemmae occur only on the upper surface of the thickened nerve in the apical half of the young leaves, and are attached by means of a thin stalk which still adheres to the gemma when it becomes separated from the leaf. In *T. mutica*, on the other hand, the gemmae were never observed on the midrib, but grew indiscriminately over the whole of the surface of the leaf-blade, and were found on both old and young leaves, and also on the protonema. Their stalk-cell was broad and comparatively shallow, and no part of it could ever be detected on those gemmae that had become detached from the moss-plant.

I wish to thank Professor G. S. West for providing for this examination the material which had been collected by Mr. E. Cleminshaw.

B. MURIEL BRISTOL.

BOTANICAL DEPARTMENT,  
UNIVERSITY OF BIRMINGHAM.

<sup>1</sup> Loc. cit., Tab. XXXII, E. I and II.

## NOTICE OF BOOK

*Life and Letters of Sir Joseph Dalton Hooker, O.M., G.C.S.I.* By LEONARD HUXLEY. London: John Murray.

THE appearance of 'The Life and Letters of Sir Joseph Dalton Hooker', by Leonard Huxley, is an event which concerns the botanical world so nearly as to warrant a deviation from the policy, now for many years adopted by this Journal, of excluding notices of current literature from its pages. An even more intimate reason for an exceptional procedure on the present occasion lies in the fact, perhaps not generally known, that Sir Joseph Hooker was one of those who contributed greatly to the right starting of the 'Annals of Botany'.<sup>1</sup>

The life of Hooker is largely bound up with the great advance of biological science during the latter half of the last century, and his own scientific eminence, as well as his official position at Kew during a very critical period, invests with peculiar interest the full account of his life which is set forth in Mr. Huxley's two volumes. Vivid pictures are drawn of the contests waged in those early days with official stupidity and meanness, and of efforts, often made in vain, to convince those responsible for guiding the destinies of this country, its Imperial and Colonial expansion notwithstanding, of the importance of botany as a serious factor in material progress. The story of the yet more fundamental and world-wide struggle, culminating in new measures of intellectual freedom, as well as that wider outlook on life which was opened up by the new conception of the origin of species, has more than once been told. For the philosophical historian the correspondence between Hooker and Darwin, which forms no inconsiderable part of the Letters, must always possess a special value and significance. Some of the earlier incidents in the great evolution campaign acquire new meaning, and others were unfolded for the first time. Hooker's great wealth of knowledge served usually to reinforce the position of tenacious criticism which he ever maintained towards unproved inferences and unsubstantiated hypotheses. It also provided a powerful instrument in forging the new weapons with which Darwin was to shatter old dogmas concerning the constancy of species, and thence to bring about the greatest philosophical upheaval the modern world has ever known.

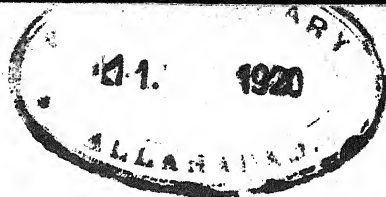
This, however, is not the place to attempt to analyse Hooker's contributions to the edifice of Science, however important they are, or indeed to dwell upon them in detail. Others have already discussed these things in many journals and reviews. It is the place to indicate emphatically that the 'Life and Letters' is a book that deserves to be read by all botanists, and especially perhaps by the younger generation. Hooker's was no life of ease. It is the story of strenuous effort and continuous

The writer is indebted to Professor I. Bayley Balfour, to whose energy and foresight the idea and ultimate realization of the *Annals of Botany* was mainly due, for access to Hooker's letters during the pre-natal period of its development.

work crowned by great achievement. Mr. Huxley has treated his subject with admirable tact. It is Hooker himself, through his letters, who is telling the story of his life. And in this self-revelation we seem to gain an intimate knowledge of the man himself, ever modest—almost to a fault—and endowed with a nobility of character which would have ensured for him an honoured position, whatever the walk of life he might have marked out for his own.

It is impossible to read the 'Letters' without being struck by the wisdom, founded as it was on natural shrewdness and accumulated experience, which made Hooker's advice and criticism so valuable to his friends and colleagues. He was not always easy to convince at the outset, and this quality of caution, which had served him in good stead both in scientific and official matters, again found expression when the inception of the 'Annals of Botany' was under discussion. But no sooner had he assured himself that the enterprise deserved his support than he rendered it with his whole heart. It is safe to say that some day, when the early history of this Journal comes to be written, the share taken by Hooker in launching the new enterprise on the botanical world will be seen to have been no small one.





# The 'Brown Rot' Diseases of Fruit Trees, with Special Reference to two Biologic Forms of *Monilia cinerea*, Bon.<sup>1</sup> II.

BY

H. WORMALD, M.Sc. (Lond.), A.R.C.Sc.

*Mycological Department, South-Eastern Agricultural College, Wye, Kent.*

With Plates IV and V.

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## I. INTRODUCTION.

IN Part I of this article it was shown that the 'Brown Rot' diseases of fruit trees in this country are caused not only by *Monilia fructigena*, which has been generally assumed to be the species responsible for all such diseases in Britain, but also by *M. cinerea*, the latter having been found, in many cases, to be more destructive than *M. fructigena*; the two species are to be recognized by their morphological characters and by their mode of parasitism. Further, it was pointed out that the morphological species *M. cinerea* includes two 'biologic' forms distinguished by the degree of virulence when apple flowers are inoculated with their conidia; one form, found on infected flowering shoots and cankers of apple trees, is able to

<sup>1</sup> This article is included in a Thesis approved for the Degree of Doctor of Science in the University of London.

produce a blossom wilt and cause cankers on the branches by infection through the flowers; the other kills only the flower actually inoculated, infection extending no farther than the pedicel of the infected apple flower.

In the following pages experiments are described which show that the two biologic forms of *Monilia cinerea* can be distinguished in the laboratory by biochemical methods. The value of the application of cultural methods to taxonomy, as illustrated by certain distinguishing characters exhibited by *M. fructigena*, *M. cinerea*, and an American form of *Monilia* when grown in pure cultures, is also discussed.

## II. THE SECRETION OF AN OXIDIZING ENZYME BY *MONILIA CINEREA*.

When growing on agar, prepared with an extract of prunes as its nutrient constituent, at room temperature (about 18° C.), the apple Blossom Wilt strains of *Monilia cinerea* invariably give rise to a dark brown coloration. Strains from other sources developed this browning in varying degrees; a few remained quite hyaline on that medium or produced merely a slight coloration in some cultures, while with others the colour was quite as intense as that of the apple strains. The fact that chromogenesis in fungi is often due to the action of oxidizing agents elaborated by the organisms suggested the presence of an enzyme (probably an oxidase) in the *Monilia* cultures, and experiments were initiated with the object of ascertaining whether such an enzyme could be detected and identified. The results, though they showed no close correlation with the coloration of the agar cultures, proved to be of particular interest in relation to the inoculation experiments on apple flowers described in Part I.

### (a) *The Enzyme in Liquid Culture Media.*

Preliminary experiments were carried out with two strains used in the inoculations of apple flowers made in 1917, viz. an apple Blossom Wilt strain, and one obtained from a vegetative shoot of a plum tree affected with the 'Wither Tip' disease. The former, in common with all other strains obtained from apple spurs and cankers, developed a dark brown coloration on prune extract agar, while the latter in a few cultures produced a little browning, but generally remained quite hyaline. The Wither Tip strain had failed to produce a blossom wilt when apple flowers were inoculated with it.

In the first experiment of this series the two strains, here referred to for convenience as A (apple strain) and B (plum strain) respectively, were grown at room temperature in Petri dishes containing a sterilized extract of apples. After 16 days the liquids were poured from the dishes into wide test-tubes, when it was seen that in both cases they were darker than the

original culture medium and that the liquid from A was distinctly darker than that from B.

Four c.c. of emulsified gum guaiacum were poured into each of two test-tubes; to one tube was added 1 c.c. from culture A and to the other 1 c.c. from B. At the end of 30 minutes the contents of the first tube were a bright blue, while the latter was unchanged, though a very faint bluish tint could be detected after 4 hours. Two other tubes similarly prepared shortly afterwards gave a similar result. When either liquid was heated in a water-bath at 100° C. for 2½ minutes, before adding the emulsion, no trace of the blue coloration appeared.

The mycelium from these cultures was washed with distilled water, dried in a thermostat at 30° C., cut up into small pieces, and stored in stoppered bottles until required. 0.1 gm. of the dried mycelium of each strain was weighed out, soaked in a few c.c. of water, and pounded in a mortar to obtain an extract which was finally made up to 50 c.c. These extracts were tested for oxidase in the same manner as employed for the culture media. In this case the plum strain gave the reaction for oxidase, but not the apple strain.

The results go in the direction of showing that both strains produce the oxidase, but that the apple strain secretes it more readily into the culture medium.

In another experiment, using the same two strains, similar plate cultures were prepared, eight in number, viz.:

A 1, A 2, A 3, A 4, inoculated with the apple strain;  
B 1, B 2, B 3, B 4, „ „ „ plum „

Of these Nos. 1 and 2 of each set were incubated at 25° C., while Nos. 3 and 4 were kept at room temperature (about 18° C.). At the end of 9 days Nos. 2 and 4 of both sets were tested for the presence of oxidase in the liquid, with the following colour changes in the test-tubes at the end of 1 hour:

A 2, deep blue;  
A 4, dark blue;  
B 2, very faint bluish tint;  
B 4, no colour change.

Seven days later the remaining four plates were similarly tested:

A 1, deep blue;  
A 3, deep blue;  
B 1, very faint bluish tint;  
B 3, no colour change.

It will be seen that the apple strain gave approximately identical results at the end of 9 and of 16 days and whether incubated or kept at room temperature; the liquid in which the plum strain had been growing, however,



gave no oxidase reaction in the culture grown at the lower temperature and only a very faint reaction in the one at the higher.

In this and subsequent experiments it was generally found that the colour reaction in the test-tubes reached its greatest intensity in 2 to 3 hours, after which the colour gradually faded, but often a trace remained after 24 hours.

Cultures were also grown in flasks containing 200 c.c. of the apple extract, kept at room temperature, so that portions of the culture medium could be removed at intervals of a few days and subjected to the oxidase test.

The strains used in this experiment were :

- A. The apple Blossom Wilt strain used in the preceding experiment.
- B. The plum Wither Tip strain used in the preceding experiment.
- C. } Two other Wither Tip strains.
- D. }
- E. Strain of *Monilia* obtained from America.

By means of a sterile pipette 10 c.c. of the culture medium were removed from each flask at intervals of 9, 15, 37, and 58 days from the time the cultures were started, and the guaiacum test applied. The maximum colour change given by the five strains was as follows:

Age of culture.	Reaction to Guaiacum Test.				
	A.	B.	C.	D.	E.
9 days	pale blue	no change	no change	no change	deep blue
15 days	" "	very pale blue	" "	" "	very deep blue
37 days	deep blue	" "	" "	" "	" "
58 days	rather pale blue	deep blue	very pale blue	very pale blue	rather pale blue

The results show that although the plum strains are capable of secreting the oxidase into the culture medium, the enzyme is set free far more readily by the apple strain and the American form of *Monilia* during the period of vigorous growth.

The colour of the culture medium (apple extract) in which the fungi had been growing for some time was generally darker than before inoculation, and the depth of colour was correlated with the intensity of the oxidase reaction given by the liquid. Thus the liquid of the cultures of the apple strains and of the American form of *Monilia* was invariably darker than those of the plum strains, and proved to give a more intense reaction to the oxidase test.

Experiments carried out with the object of identifying the oxidase showed that it had no action on tyrosin or hydroquinone; it could not therefore be referred either to tyrosinase or laccase, two oxidases which have been found in fungi. On the other hand it rapidly produces a brownish yellow colour in solutions of tannic acid, gallic acid, and pyrogallie acid. In this respect it behaves as the enzyme oenoxydase found by Martinand (16, 17) in ripe grapes and ascertained by Laborde (14) to be secreted by

*Botrytis cinerea* when growing on grapes and on sterilized wine 'must'. This enzyme, however, is described as oxidizing hydroquinone, while in repeated experiments with the oxidizing enzyme of *Monilia cinerea* no action on hydroquinone could be detected.

Martinand found that oenoxylase is destroyed by heating to 72° C. and keeping it at that temperature for four minutes. The *Monilia* enzyme also loses its power of oxidation when similarly heated. In most of the experiments described below the control tubes were heated for two minutes in a water-bath at 100° C. In one experiment, however, the temperature of the bath was 72° C., when the tubes were plunged in and kept at that temperature for four minutes; on cooling the tubes and applying the guaiacum test no reaction occurred, while in corresponding unheated tubes a vigorous reaction was soon evident.

The action of the enzyme tannase (secreted by *Aspergillus niger* and *Penicillium* spp.), which has been investigated by Fernbach (10), Pottevin (22), and Knudson (13), is one of hydrolysis, tannic acid being hydrolysed to gallic acid. The enzyme secreted by *Monilia cinerea* is therefore quite different from tannase, and is probably more nearly related to the oxidase found by Thatcher (25) in apples and that described by Crocker and Harrington (7) as occurring in the 'seeds' of certain grasses.

The experiments just described suggested the procedure finally adopted in investigating the oxidizing activities of the strains of *Monilia* which had been isolated and cultivated, a comparative study of those strains which had been used in the inoculation experiments on apple flowers carried out in 1918 being particularly desirable. The general method employed in this series of experiments was one which eliminated as far as possible differences due to variations in environmental factors and in manipulation. Thus throughout the series the following points were observed:

1. The same nutrient medium was used for all the experiments with the exception of Expt. 8.
2. The cultures to be tested were started from vigorously growing mycelium taken from the edge of young agar cultures.
3. The cultures were grown in Petri dishes 8.5 cm. in diameter (internally) and 20 c.c. of the culture liquid were used in each.
4. The cultures were incubated at 25° C. for seven days, the oxidase test being applied on the seventh day.

The culture medium was an extract of dried peaches. The apple extract used in previous experiments was rejected because of the coagulation of the pectin under the action of the fungi, the coagulum rendering the pipetting of small quantities of the liquid troublesome and inaccurate. Uschinsky's solution and a synthetic medium which Coons (6) found to be suitable for *Plenodomus fuscomaculans* both gave far less vigorous growth than fruit extracts in the case of the *Monilias*. Prunes, which had been

generally used as a useful nutrient substrate for agar cultures, were unobtainable at the time the experiments were started, but dried peaches were available and these gave good results.

Two grammes of the dried fruit were weighed out for every 100 c.c. of the liquid required, cut up into small pieces, and extracted with 100 c.c. of distilled water in a steam sterilizer at 100° C. for one hour. The extract was filtered and transferred to test-tubes, 20 c.c. to each, which were then plugged with cotton-wool and sterilized by heating at 100° C. for 20 minutes on each of three successive days. The Petri dishes were sterilized by dry heat at 160° C. for one hour. When cultures were to be started the contents of the tubes were emptied, with the usual precautions to avoid contamination, into the respective dishes; these were inoculated by transferring to each a small portion of the mycelium from the edge of a vigorously growing agar culture. The dishes were then placed in a thermostat at 25° C. Thus the conditions under which the cultures were grown were the same for all the experiments, and a comparison could be made of the behaviour of strains cultivated at different times. Each experiment consisted of the culture and subsequent testing for oxidase of from 4 to 7 strains, both apple and *Prunus* strains being included in every experiment. After 7 days' growth the liquid contents of each plate were poured off into wide test-tubes, from which the liquid was withdrawn as required for the oxidase tests.

Reynolds Green (11) says, in reference to the guaiacum test, 'most investigators do not find it give entirely satisfactory results'. Woods (26), however, states 'that with proper care it is the best reagent which we have' for the detection of oxidizing enzymes. It has been used throughout this series of experiments, but, with the exception of No. 1, the results have always been checked and confirmed by testing simultaneously for the reaction with pyrogallic acid. The guaiacum emulsion was prepared by soaking 10 grm. of gum guaiacum in 100 c.c. of 95 per cent. alcohol, with occasional shaking, for several days before using; 5 c.c. of this tincture were shaken up with 95 c.c. distilled water to make 100 c.c. of the white emulsion, which was prepared fresh for each experiment. The pyrogallic acid was used as a 2 per cent. solution prepared immediately before use.

The following details of Expt. 6 (see table on page 151) of this series will serve as an example of the method adopted in applying the tests.

#### EXPERIMENT 6.

Four strains were used in this experiment:

- A. From an apple spur.
- B. From an apple canker.
- C. From a mummied cherry.
- D. Strain from Oregon.

When the cultures were seven days old the liquid was poured off into wide test-tubes labelled to correspond with the strains. For each strain six narrow tubes (1 cm. internal diam.) were prepared and numbered 1 to 6. Into each of Nos. 1, 2, and 3 was run (with a graduated pipette) 1 c.c. of the culture medium and into Nos. 4, 5, and 6 were run 2 c.c. of the medium; the colour change in the pyrogalllic acid not being so striking as in guaiacum emulsion, the larger volume was used for this reagent. Tubes 3 and 6 were next heated in a water-bath at 100° C. for two minutes and allowed to cool. Into tubes 1, 2, and 3 were now run 5 c.c. of the guaiacum emulsion and into 4, 5, and 6 five c.c. of the 2 per cent. pyrogalllic acid solution. The tubes were then placed in a thermostat at 25° C., examined from time to time, and the colour changes noted. In the guaiacum emulsion the change in colour corresponded very closely to the series of tones in Ridgway's colour scheme (23) on Plate XXXV, 49" Blue, and that series was used in naming the colours.

(a) Guaiacum Emulsion as Reagent.

Results in tubes 1 and 2.

Time.	A.	B.	C.	D.
10 min.	no change	noticeable	almost pearl blue	no change
30 min.	almost pearl blue	deeper than pearl blue	ditto	pearl blue
1 hour	deeper than pearl blue	pale Windsor blue	ditto	pale Windsor blue
2 hrs.	deeper than pale Windsor blue	almost light Windsor blue	ditto	almost light Windsor blue
3 hrs.	light Windsor blue	ditto	very slight tinge of blue	ditto
5 hrs.	ditto	ditto	ditto	ditto
10 hrs.	lighter than pale Windsor blue	lighter than pale Windsor blue	ditto	pearl blue
24 hrs.	lighter than pearl blue	lighter than pearl blue	colour a little more distinct but still very faint	ditto
48 hrs.	almost decolorized	almost decolorized	very faint tint	almost decolorized

Tube No. 3 in each case remained unchanged throughout the experiment.

(b) 2 per cent. Pyrogalllic Acid as Reagent.

Results in tubes 4 and 5.

Time.	A.	B.	C.	D.
10 min.	no change	no change	no change	very slightly yellower than tube 6
30 min.	very slightly yellower than tube 6	distinctly yellower than tube 6	not distinguishable from tube 6	distinctly yellower but still very pale
1 hour	very pale yellow	pale yellow	ditto	colour more distinct
2 hrs.	pale yellow	colour more distinct	ditto	ditto
3 hrs.	ditto	bright yellow	ditto	bright yellow
5 hrs.	bright yellow	ditto	ditto	ditto
10 hrs.	ditto	ditto	ditto	ditto
24 hrs.	deep brownish yellow	very deep brownish yellow	ditto	very deep brownish yellow
48 hrs.	ditto	ditto	ditto	ditto

All the tubes at first showed a very faint yellowish tint owing to the colouring matter present in the culture medium. Tube No. 6 of each strain showed a very gradual deepening of the tint due to the slow oxidation of the pyrogalllic acid, which normally darkens when in solution in contact with the air, but after 24 hours all the four tubes were still a very pale yellow and the tint was but slightly deeper after 48 hours.

The following table gives a resumé of the results obtained for all the strains of *Monilia* tested in this series of experiments. The numbers indicate the maximum colour change which developed in each case; the values of these numbers in the guaiacum test are as follows:

- |    |                                |
|----|--------------------------------|
| 1. | indicates a faint bluish tint. |
| 2. | corresponds to pearl blue.     |
| 3. | „ pale Windsor blue.           |
| 4. | „ light Windsor blue.          |
| 5. | „ clear Windsor blue.          |

The colour changes in the pyrogalllic acid could not be so readily defined, the transparent liquids not being suitable for use with the colour chart. 1, however, indicates a very slight yellowing just discernible when the tubes were carefully examined side by side with the control (heated) tube, while 5 denotes a deep brownish yellow. In Expt. 1 tannic acid was used instead of pyrogalllic acid.

As in the case of the apple extract, it was found in these experiments that in general the darker the culture medium (extract of peaches) had become under the action of the particular strain growing in it, the more intense was the oxidase reaction it gave. The cultures in Coons' solution, however, all remained colourless, and as the results obtained in the tests, though essentially confirming those obtained with the fruit extracts, showed some variation in detail from the rest of the series, the results are given fully.

Comparative results of tests for presence of oxidase (reagents: tincture of guaiacum and pyrogallol acid) given by strains of *Monilia cinerea* in cultures 7 days old growing at a temperature of 25°C.

Sources from which the strains were obtained.		Culture medium: extract of dried peaches (2 %).										Coon's solution.					
		Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5		Exp. 6		Exp. 7		Exp. 8	
		Gua.	Tan.	Gua.	Py.	Gua.	Py.	Gua.	Py.	Gua.	Py.	Gua.	Py.	Gua.	Py.	Gua.	Py.
Strains from apple trees:																	
(1)	*Apple canker (Berks.), 1916.	...	...	...	...	...	...	...	...	4	3	...	...	3	3	...	...
(2)	*Strain (1) re-isolated from apple spur, 1917.	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(3)	*Strain (1) re-isolated from apple spur, 1918.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(4)	*Apple twig (N. Kent), 1916.	...	...	4	3	...	...	...	...	...	...	...	...	...	...	...	...
(5)	Young apple (Weald of Kent), 1916.	...	...	4	3	...	...	...	...	...	...	...	...	...	...	...	...
(6)	Cankered apple twig (Ireland), 1916.	...	...	...	...	...	...	5	4	...	...	...	...	...	...	...	...
(7)	Cankered branch (Weald of Kent), 1917.	...	...	...	...	...	...	5	4	...	...	...	...	...	...	...	...
(8)	*Dead spur (Wye), 1918.	...	...	...	...	4	3	...	...	4	4	...	...	...	...	4	4
(9)	Cankered branch (Norwich), 1918.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Strains from other sources:																	
(10)	Mummied damson (Wye), 1915.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(11)	Mummied damson (Wye), 1915.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(12)	* <i>Pyrus japonica</i> (Mid-Kent), 1917.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(13)	*'Wither Tip' of plum (Wye), 1917.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(14)	*'Wither Tip' of plum (Wye), 1918.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(15)	*'Wither Tip' of plum (Wye), 1916.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(16)	*'Wither Tip' of plum (Wye), 1916.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(17)	Cherry flower (E. Kent), 1915.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(18)	*Mummied cherry (Mid-Kent), 1918.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(19)	*Mummied plum (Cambridge), 1918.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(20)	Mummied plum (Worcester), 1918.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(21)	Mummied plum (Devonshire), 1918.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(22)	Mummied cherry (N. Kent), 1918.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Strains from America:																	
(23)	Apple (Oregon), 1915.	...	...	2	2	...	...	...	...	...	...	...	...	...	...	2	4
(24)	Apple (Oregon), 1915.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
(25)	Peach (Ontario), 1916.	...	...	...	...	...	...	...	...	...	...	5	4	...	...	...	...
(26)	Plum (Ontario), 1916.	...	...	...	...	...	...	...	...	...	...	4	3	...	...	...	...
(27)	Peach (Wisconsin) 1916.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	5
(28)	Peach (Ontario), 1917. Strain from ascospore	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	4
(29)	Plum (Ontario), 1917. Strain from ascospore	...	...	...	...	2	2	...	...	...	...	...	...	...	...	...	...
(30)	Plum (Ontario), 1917. Strain from ascospore	...	4	2	...	...	...	...	...	...	...	...	...	...	...	...	...

The strains marked \* were those used for the inoculation experiments on apple flowers as described in Part I of this article. In every case the strains which produced the 'Blossom Wilt' responded readily to the oxidase tests; those which failed to infect the flowering axis gave either no oxidase reaction or a very feeble one.

## EXPERIMENT 8.

*Strains grown in Coons' Solution.*

- A. From an apple spur.
- B. From a mummied cherry.
- C. From a plum twig affected with the 'Wither Tip' disease.
- D. A strain from America (Oregon).
- E. Another strain from America (Ontario).

*(a) Guaiacum Emulsion as Reagent.*

Results in tubes 1 and 2.

<i>Time.</i>	<i>A.</i>	<i>B.</i>	<i>C.</i>	<i>D.</i>	<i>E.</i>
2 hrs.	slight bluish tint	no change	no change	no change	pearl blue
4 hrs.	deeper than pearl blue	ditto	ditto	ditto	clear Windsor blue
8 hrs.	deeper than pale Windsor blue	ditto	ditto	ditto	ditto
24 hrs.	light Windsor blue	ditto	ditto	pearl blue	ditto

*(b) 2 per cent. Pyrogallie Acid as Reagent.*

<i>Time.</i>	<i>A.</i>	<i>B.</i>	<i>C.</i>	<i>D.</i>	<i>E.</i>
2 hrs.	very pale yellow	no change	no change	no change	very pale
4 hrs.	pale yellow	ditto	ditto	very pale yellow	pale yellow
8 hrs.	ditto	ditto	ditto	ditto	ditto
24 hrs.	bright yellow	ditto	very pale yellow	bright yellow	deep yellow

In Coons' solution the growth of the strains was comparatively feeble, the diameter of the mycelial discs at the end of seven days being only about half that of the cultures of the same age in the fruit extracts, and the hyphae were less densely interwoven. The oxidizing reaction of the cultures was more gradual than in the other experiments of the series, and the pyrogallie acid proved to be the more sensitive of the two reagents.

The dark coloration developed in the fruit extracts when certain strains of *Monilia cinerea* are growing in them is, in all probability, due to the oxidation of tannins, present in the extracts, by the oxidase secreted by the fungus. That the presence of tannins is not a factor necessary for the secretion of the oxidase is shown by the cultures grown in Coons' solution, a medium consisting of inorganic salts, asparagin, and glucose.

It was found in every experiment that the strains of *Monilia cinerea* from apple trees produced the oxidase far more readily than those from other sources, with the exception of the American strains, which appear to belong to a distinct variety, if not species. This is correlated with their power of infecting apple flowers; five of the strains which have given no oxidase reaction, or a very feeble one, have been used in the inoculation experiments on apple flowers, and all failed to produce the blossom wilt condition which is typical of infection by the apple strains.



(b) *The Enzyme in Infected Apples.*

It has already been shown (80) that infection of apples with an apple strain and with a plum strain of *Monilia cinerea* gave different results, the latter causing a brown rot, while with the former the brown colour gradually assumed a darker tone and eventually became black. The enzyme experiments just described suggested that the differences might be due to the oxidase secreted by the apple strain. To verify this the following experiments were made:

EXPERIMENT 1.

On October 26, 1917, four apples were inoculated, two with an apple strain and two with a Wither Tip strain from a plum tree. On Nov. 30 the former were black, the latter a bright brown. From each of the four apples a cube of tissue (side of cube 5 mm.), including the skin, was cut out at 1.5 cm. from the point of inoculation and ground up with a few c.c. of distilled water in a mortar; more water was then added to make up to 6 c.c., and 3 c.c. were poured into each of two test-tubes. 2 c.c. of guaiacum emulsion were added to each tube and all were incubated at 30° C. Two control tubes were also prepared from a cube of tissue similarly cut from a sound apple and extracted.

The oxidase reaction was greatest in the tubes containing the extract of the apple infected with the apple strain; the other tubes (including the controls) also gave the reaction, but the colour change was feebler.

EXPERIMENT 2.

The strains were those used in Expt. 1. An apple was inoculated on Nov. 28 on one side with the apple strain, and on the other with the plum strain. By Dec. 11 both strains had caused a discoloration of the surface, but there was still a strip of healthy tissue midway between the two infected areas. Six cubes of tissue (side of cube 5 mm.) were cut out of the apple, viz.:

(1) from the side infected with apple strain, at 1 cm. from the edge of the discoloured area;

(2) as (1), but at 2 cm. from edge of discoloured area;

(3) from the side infected with plum strain, at 1 cm. from the edge of the affected area;

(4) as (3), but at 2 cm. from edge;

(5) } from the healthy portion of the same apple.  
(6) }

Each cube of tissue was ground in a mortar with a little distilled water, and more water was then added to make up to 5 c.c.; this was divided into

two parts by pouring 2.5 c.c. into each of two test-tubes. 10 c.c. of the guaiacum emulsion were added to each tube. Result:

Time.	From side infected with apple strain.		From side infected with plum strain.		From healthy portion.	
	(1) 2 tubes	(2) 2 tubes	(3) 2 tubes	(4) 2 tubes	(5) 2 tubes	(6) 2 tubes
1 hr.	bright blue	bright blue	pale blue	very pale blue	no change	no change
2 hrs.	deep blue	deep blue	ditto	pale blue	ditto	ditto
10 hrs.	ditto	a little paler	nearly decolorized	very pale blue	ditto	ditto
20 hrs.	very pale blue	very pale blue	decolorized	decolorized	ditto	ditto

### EXPERIMENT 3.

This was carried out as in Expt. 2, using another apple similarly inoculated. The results were essentially the same as those of Expt. 2; the control tubes (containing extract of sound tissue) again gave no oxidase reaction, the tubes of the apple strain gave a vigorous reaction, and those of the plum strain a feeble one (see Fig. 8).

### EXPERIMENT 4.

From the same apple as used in Expt. 2 cubes of tissue were cut from infected and healthy portions and extracted, but instead of guaiacum a 2 per cent. solution of tannic acid was used as the reagent. Again the reaction was greatest in those tubes containing the extract of tissue infected with the apple strain, slight in those with the plum strain, and not discernible in the control tubes.

The absence, in these experiments, of any reaction in the tubes containing the extract of the uninfected portions of the apples calls for remark, since Lindet (15) and Thatcher (25) find that apple juice contains an oxidase which acts on tannins. It is clear, however, that, in the experiments recorded above, if an oxidase were present in the tissues of the apples its action was so feeble that it could not be detected by the method adopted, but this in no way affects the comparative results, which show that an active oxidase was secreted by the apple strain of *Monilia cinerea* when parasitic within the tissues of the apples.

The conclusion is that the blackening of the apples infected with *Monilia cinerea* forma *mali* is correlated with the secretion by the fungus of an enzyme which oxidizes tannin, and the nigrescence is probably the result of the oxidation of tannins in, or immediately below, the skin.

### (c) *The Enzyme in Infected Apple Spurs.*

The preceding experiments, which show that the 'Blossom Wilt' *Monilia* of apple trees produces in liquid culture media and in infected apples an enzyme which rapidly oxidizes tannins, suggested that the same

enzyme might be secreted when the fungus is invading the tissues of apple spurs. Experiments were therefore carried out with the object of determining whether that or a similar enzyme could be detected in infected spurs.

#### EXPERIMENT 1.

In this preliminary experiment a dead spur showing typical blossom wilt was obtained from a Lord Derby apple tree in the College plantation; the current year's growth (which was not yet fully lignified and therefore softer than the woody portion of the previous year) was cut off and the dead leaves and flowers removed. This short shoot, about 1.5 cm. in length, was pounded with about 1 c.c. of water and ground in a mortar to a paste, which was washed out with a little more water into a test-tube and finally made up to 5 c.c. This extract was allowed to stand for a few minutes until the grosser particles sank to the bottom; the liquid above was then poured off and 1 c.c. of it was transferred by means of a pipette to each of three test-tubes. One tube was heated in a water-bath at boiling-point for two minutes and cooled; then to each of the three tubes were added 5 c.c. of guaiacum emulsion and the tubes were placed in an incubator at 25° C. Tubes 1 and 2 (unheated) gradually assumed a bluish tint, and after three hours were a pale blue; in the third tube no change occurred. It appeared evident that an oxidase could be extracted from an infected spur, so other experiments were carried out to ascertain whether this enzyme was similar in action to that secreted by the fungus when growing in liquid culture media, and also whether it occurred exclusively in infected spurs.

#### EXPERIMENT 2.

An extract was obtained from another infected spur as in Expt. 1 and a normal (living) spur was also extracted for comparison. Three tubes of each extract were prepared as in the previous experiment, one of the three being heated before adding the guaiacum emulsion, and all were placed in the thermostat.

The emulsion rapidly became coloured in the tubes containing the extract of the living spur, but the colour was much greener than in those tubes containing the infected material; the latter were coloured more slowly, but eventually were approximately of the same tone as that obtained when using the liquid from pure cultures.

The contrast between the two sets of tubes was very marked after 24 hours:

Extract of infected spur (unheated) and guaiacum emulsion: bright blue (approx. 'Russian Blue' of Ridgway's scheme).

Extract of normal spur (unheated) and guaiacum emulsion: bright green (approx. 'Deep Grape Green').

Both tubes containing the heated extract remained unchanged.

## EXPERIMENT 3.

This was a repetition of Expt. 2, using two other spurs—one infected and the other normal. The result was practically the same.

## EXPERIMENT 4.

This was carried out as Expts. 2 and 3, except that the spurs were taken from another variety of apple tree, viz. James Grieve, those used in the previous experiment having been taken from a tree of the Lord Derby variety; the method, too, was slightly modified, the extracts being prepared as before and then centrifugalized to give a clearer solution.

The result again was as before.

## EXPERIMENT 5.

Extracts were prepared of two infected and two normal spurs and centrifugalized; those of one infected and one normal spur were filtered through Swedish filter-paper. The extracts were then tested for their reaction with the guaiacum emulsion and with pyrogalllic acid. Since the slight turbidity of the centrifugalized extracts was not removed by filtering, and as the results obtained with filtered and unfiltered extracts of similar spurs were identical, the filtering was not repeated in subsequent experiments.

Tubes of each of the four extracts were prepared, 1 c.c. of the extract being placed in each, with 5 c.c. of the reagent, viz.:

1. Guaiacum emulsion.
2. " " control (i.e. the extract being previously heated).
3. 2 per cent. pyrogalllic acid.
4. " " "
5. " " " control (extract heated).

Result in unheated tubes:

Time.	Guaiacum emulsion.		Pyrogalllic acid.	
	Infected spur.	Normal spur.	Infected spur.	Normal spur.
4 hrs.	bright blue	no change	no change	very pale yellow
24 hrs.	pale blue	pale buff yellow	deep yellow	ditto

The control tubes of guaiacum emulsion remained unchanged; a slight yellowing of the pyrogalllic acid control tubes was the result of the usual oxidation of the acid in solution when in contact with the air.

Expts. 6 and 7 were carried out as in Expt. 5, except that the filtering was omitted, and that the infected spurs used were those which had been infected by artificial inoculation from pure cultures of apple Blossom Wilt strains which are included among those that have been proved to produce the enzyme when growing in liquid culture media (Strains 1, 2, 3, and 8 of table on page 151).

The results were the same as those in Expt. 5, except that the extracts of normal spurs gave a more distinct yellow colour to the pyrogallio acid solution.

It will be observed that in the earlier experiments of this series (Expts. 1-4), which were carried out from May 24 to 28, the extracts of normal spurs invariably produced a bright green coloration of the guaiacum emulsion, while later experiments (5-7), carried out from May 29 to June 7, failed to produce the green coloration of the emulsion and extracts of normal spurs gave only a buff yellow. It seemed probable that this yellow colour was due to a change occurring in the extract itself which imparted a tint to the mixture without any reaction with the emulsion. In this case the green colour of the earlier experiments might have been the result of an oxidase reaction with the emulsion to produce a blue which, mixed with the yellow extract, would give a green.

That the green colour can be reproduced in this way was shown by Expts. 8 and 9.

#### EXPERIMENT 8.

- A tube was prepared by mixing
- 1 c.c. of an extract of an infected spur,
  - 1 c.c. of an extract of a normal spur,
  - 5 c.c. guaiacum emulsion.

After incubating for 4 hours at 25° C. the contents of the tube were a bright green.

#### EXPERIMENT 9.

Three tubes were prepared as follows :

- (1) 1 c.c. of extract from infected spur + 1 c.c. distilled water.
- (2) 1 c.c. extract from infected spur + 1 c.c. extract of normal spur.
- (3) 1 c.c. extract normal spur + 1 c.c. distilled water:

5 c.c. of guaiacum emulsion were added to each and the tubes were incubated. At end of 20 hours the contents were

- (1) pale blue;
- (2) bright green;
- (3) buff yellow.

In order to ascertain whether the yellow pigment which was produced in the extracts of normal spurs could be detected in extracts of infected spurs, another experiment was carried out.

#### EXPERIMENT 10.

Extracts were made of infected and normal spurs and centrifugalized; each was divided into two portions, one of which was heated for one minute in a water-bath at 100° C. and the other left unheated. The tubes were then incubated without the addition of any reagent.

Result :

		<i>Extract of infected spur.</i>		<i>Extract of normal spur.</i>	
		<i>Unheated.</i>	<i>Heated.</i>	<i>Unheated.</i>	<i>Heated.</i>
4 hrs.	no change		no change	deep yellow	no change
24 hrs.	ditto		* ditto	ditto	very pale yellow

The result was confirmed in three other similar experiments. The freshly made extracts, after being centrifugalized, were in each case almost colourless, but with slight turbidity.

#### EXPERIMENT II.

In this experiment the action of the enzyme in an infected spur on the chromogenic substance of the normal spur was examined. A normal spur was extracted with 10 c.c. distilled water ; this extract was centrifugalized and then heated in a water-bath for 5 minutes. Two tubes were set up as follows :

- (1) 5 c.c. of this extract + 1 c.c. of unheated extract of an infected spur.
- (2) 5 c.c. of this extract + 1 c.c. of heated extract of an infected spur.

The tubes were incubated at 25° C. At the end of 8 hours the contents of both tubes were distinctly yellow, but the liquid in (1) was of a deeper colour than that of (2).

The experiment was duplicated, using two other spurs, and a similar result was obtained.

It would seem, then, that an extract of a normal spur contains a substance which darkens in colour on contact with air, but that the process is hastened by the enzyme (found in infected spurs) secreted by the fungus.

The results of these experiments lead to the following conclusions :

(1) That in the extract of a normal (healthy) flowering spur of apple trees there is present some substance, probably a tannin, which assumes a deep yellow colour when the extract remains in contact with the air. That this colour change is in part due to enzyme action is suggested by the fact that the action is retarded when the extract has been previously heated, but such an enzyme, if present, gives no reaction with guaiacum. In addition there is evidence that flowering spurs also contain an oxidase (reacting with guaiacum) at about the time the flowers are setting into fruit, but that later it cannot be detected.

(2) That in an infected spur there is present an enzyme which reacts with guaiacum and oxidizes pyrogalllic acid.

(3) That the chromogenic tannic substance present in a spur is oxidized, on infection, by the enzyme secreted by the fungus, so that the centrifugalized extract of the infected spur no longer contains the tannin, which must therefore have been either assimilated by the fungus or deposited in the tissues in an insoluble form.

Thus that form of *Monilia cinerea* (referred to in this paper as forma

*malis*) which causes a blossom wilt and canker disease of apple trees secretes an enzyme, easily detected in liquid culture media, in infected apples, and in infected flowering shoots; this enzyme responds to the guaiacum test and accelerates the absorption of oxygen by tannins.

The fact that those strains of *Monilia cinerea* which in inoculation experiments were unable to cause a blossom wilt of apples secrete this enzyme far less readily suggests that the virulence of the apple form may be due to this property of secreting an enzyme capable of acting on substances present in the tissues of the host plant.

In this connexion it is to be noted that Percival (21), in his investigations on the 'Silver Leaf' disease of fruit trees, found that the disease was associated with the presence of an oxidase which is easily extracted from diseased tissues; later, however, Brooks (5) stated that 'until the present I have been unable to confirm Percival's view that the disturbing agent is an oxidase which is secreted by the fungus'.

Whether the secretion of an oxidase is a factor determining the degree of virulence of the Brown Rot fungi is yet to be determined, but the fact that two biologic forms morphologically similar are in some respects physiologically distinct under laboratory conditions is of considerable interest, and further investigations along these lines may throw some light on the mode of parasitism of such biologic forms.

### III. MORPHOLOGY AND TAXONOMY.

#### (a) Colour and Size of Pustules.

When *Monilia fructigena* and *M. cinerea* are growing under the same conditions, they can, as a rule, be readily distinguished by their conidial fructifications. Thus the pustules of the former are larger than those of the latter and are yellow in colour, while the pustules of *M. cinerea* are, as its specific name implies, ashy grey. These characters may, however, show some variation according to the conditions under which the fructifications are produced. The difference between the two is most striking when they are growing vigorously on recently infected fruit. Thus in an experiment where plums were simultaneously inoculated with the two species, *M. fructigena* produced pustules of a maximum diameter of 1.5 mm. (mostly about 1 mm.) and 'Light Buff'<sup>1</sup> in colour, while those of *M. cinerea* only reached a maximum diameter of 0.8 mm. (mostly about 0.4 mm.) and were 'Smoke Grey'<sup>1</sup> in colour. The pustules had, in this instance, developed during a period of dry weather and were on that account comparatively small.<sup>2</sup> When produced in a moist atmosphere the pustules are larger, but the relative size for the two species remains approximately the same.<sup>3</sup> Pustules of *M. cinerea* on apple spurs and cankers may under favourable conditions

<sup>1</sup> Ridgway's scheme of colours.

<sup>2</sup> Vide Part I, Figs. 8 and 9.

<sup>3</sup> Vide Part I, Fig. 2.

reach a diameter of 1.5 mm., while *M. fructigena* when growing on maturing apples and on apple spurs frequently produces pustules 2 mm. in diameter.

Old pustules of *M. cinerea* when redeveloping on mummied plums in winter and spring often coalesce, and from their general appearance at that time of the year might easily be mistaken for *M. fructigena* (cf. Figs. 2 and 3).

### (b) *Dimensions of Conidia.*

The dimensions of the conidia of the two species are generally quoted as a distinguishing character. The great variation in the size of the conidia of *M. cinerea* according to the conditions under which they are developed renders this distinction somewhat unreliable unless the environmental factors are taken into consideration. This point has been discussed in previous papers in connexion with strains occurring on apple trees and plum twigs; it was there shown that the average size of 100 conidia of each of a number of strains of *M. cinerea* found on cankers, spurs, twigs, and mummied plums was from  $11.0 \times 8.0 \mu$  to  $12.0 \times 8.5 \mu$ , but when the strains were grown on fruit in summer or on sterilized potato in the laboratory the dimensions of the conidia were about one and a half times as great.

Further observations and experiments have confirmed those results as shown in the following tables. In each case the conidia were mounted in distilled water; 100 were then measured and the average determined. To avoid unconscious selection, small groups were taken and all the conidia of each group measured, though isolated conidia occurring between the groups were also included; the range of variation was obtained by examining the whole of a slide and selecting for measurement the largest and smallest that could be found. The readings were taken correct to  $0.5 \mu$  and the averages were calculated and recorded also correct to the nearest  $0.5 \mu$ .

### Dimensions of Conidia of *Monilia cinerea*.

<i>Source of Conidia.</i>	<i>Date.</i>	<i>Range of Variation.</i>	<i>Average.</i>
Apple tree—Brown Rot canker	Mar. 8	$6.5 \times 5.0-14.0 \times 8.5 \mu$	$10.5 \times 7.5 \mu$
Apple spur	Apr. 5	$6.0 \times 4.5-15.5 \times 12.5$	$11.5 \times 8.5$
Apple tree—Brown Rot canker	June 19	$8.0 \times 6.0-19.0 \times 12.5$	$13.5 \times 9.5$
Apple twig	June 29	$8.0 \times 7.5-22.5 \times 14.5$	$14.0 \times 10.5$
Plum twig (Kent)	Mar. 14	$5.0 \times 4.0-17.0 \times 11.5$	$11.5 \times 8.5$
Mummied plum (Cambridge)	Mar. 19	$6.0 \times 5.0-19.0 \times 11.5$	$11.5 \times 8.0$
Mummied plum (Worcestershire)	Apr. 8	$5.0 \times 4.0-17.5 \times 10.5$	$11.5 \times 7.5$
Mummied plum (Devonshire)	Apr. 8	$7.0 \times 5.0-18.5 \times 11.0$	$11.5 \times 8.0$
Mummied cherry (Kent)	Mar. 4	$6.0 \times 5.0-15.0 \times 11.0$	$11.5 \times 8.0$
ditto ditto	Apr. 19	$7.0 \times 5.5-21.0 \times 15.0$	$13.0 \times 10.0$
Plum (Ireland)	Aug. 2	$10.5 \times 7.0-27.5 \times 16.5$	$18.5 \times 11.5$
Plum (Kent)	Sept. 8	$9.5 \times 7.0-23.0 \times 15.0$	$16.5 \times 9.5$
Plum (Kent)	Sept. 15	$8.5 \times 6.5-22.5 \times 18.0$	$17.0 \times 10.5$

That this difference in the average size of the conidia was due to environmental factors was shown by cultivating certain strains under various conditions and noting the variation induced by transferring any one strain from one set of conditions to another. A strain found on a mummied



cherry in March bore conidia of which the average size was  $11.5 \times 8.0 \mu$ ; when isolated and grown on a young plum in the following July the average was  $18.0 \times 13.0 \mu$ . The corresponding figures for a strain found on a mummied plum and later grown on a young plum were  $11.5 \times 8.0 \mu$  (March) and  $19.0 \times 13.5 \mu$  (July).

A strain isolated from a plum twig gave the variation in the average size of its conidia shown in the following table :

Conditions under which the Conidia were produced.	Date.	Range of Variation.	Average.
Plum twig	Mar. 27, 1917	$5.5 \times 5.0$ – $14.5 \times 9.0 \mu$	$10.5 \times 7.0 \mu$
Plum inoculated from pure culture	July 18, 1917	$10.0 \times 7.5$ – $27.0 \times 19.5$	$16.5 \times 12.5$
ditto ditto	July 31, 1917	$10.0 \times 7.5$ – $27.0 \times 14.5$	$16.0 \times 11.0$
Culture on sterilized potato in laboratory	Nov. 19, 1917	$7.5 \times 6.0$ – $25.0 \times 18.5$	$16.5 \times 12.0$
Mummied plum, result of inoculation in previous year	Feb. 28, 1918	$6.5 \times 5.0$ – $16.5 \times 11.5$	$12.0 \times 8.5$

The most convincing proof of this polymorphism of the conidia was furnished by the strain already referred to as being isolated from a Brown Rot canker of an apple tree in 1916 and used in inoculation experiments on apple flowers during three successive seasons; the results are here tabulated :

Conditions under which the Conidia were produced.	Date.	Range of Variation.	Average.
Brown Rot canker	Apr. 15, 1916	$7.0 \times 6.0$ – $14.0 \times 10.5 \mu$	$11.0 \times 8.0 \mu$
Culture on sterilized potato in laboratory	May 5, 1916	$9.0 \times 7.0$ – $26.0 \times 20.0$	$18.0 \times 13.5$
Pedicel of apple flower inoculated from pure culture in May, 1916	July 11, 1916	$9.0 \times 7.0$ – $21.5 \times 15.0$	$15.5 \times 12.0$
Dead spur, result of inoculation from pure culture in May, 1916	Feb. 13, 1917	$6.0 \times 5.0$ – $15.0 \times 10.5$	$11.0 \times 8.5$
Pedicel of apple flower inoculated May 17, 1917	May 30, 1917	$14.0 \times 11.0$ – $26.0 \times 19.5$	$18.0 \times 14.5$
Dead spur, result of inoculation in May, 1916	Mar. 22, 1918	$6.0 \times 4.0$ – $16.0 \times 11.5$	$11.5 \times 8.5$
Plum inoculated from pure culture on June 24, 1918	July 4, 1918	$9.5 \times 8.0$ – $26.5 \times 20.0$	$19.0 \times 14.0$

That the nature of the substratum on which a fungus is growing may determine the size of the conidia has been observed by Neger (19) in certain Erysiphaceae and by Brierley (4) in *Botrytis cinerea*.

The seasonal variation in the dimensions of the conidia, which is so striking in *Monilia cinerea*, is much less noticeable in the case of *Monilia fructigena*, which produces conidia freely only during the warmer months of the year. In general the conidia of *M. fructigena* are larger than those of *M. cinerea* :

#### Dimensions of Conidia of *Monilia fructigena*.

Source of Conidia.	Date.	Range of Variation.	Average.
Mummied apple, old pustules redeveloping	June 26	$11.0 \times 9.5$ – $26.5 \times 16.0 \mu$	$19.0 \times 12.5 \mu$
Plum, recently infected	July 17	$12.0 \times 8.5$ – $31.0 \times 16.0$	$21.5 \times 13.0$
Apple, " "	Oct. 25	$13.0 \times 9.0$ – $28.0 \times 16.5$	$20.5 \times 13.5$
Plum, " "	Sept. 18	$12.0 \times 9.0$ – $27.5 \times 15.0$	$20.0 \times 12.5$
Fruiting spur of apple	Aug. 29	$12.5 \times 9.5$ – $31.0 \times 17.5$	$24.5 \times 13.5$
" " "	Sept. 10	$14.5 \times 11.0$ – $29.0 \times 17.0$	$23.5 \times 14.0$

That the conidia of *Monilia fructigena* are appreciably larger than those of *M. cinerea* when produced under the same conditions was determined by measuring the conidia of the two species (1) when both were found growing on different plums of the same cluster, and (2) when both were growing on the same plum which had been inoculated on opposite sides with the two species.

Dimensions of Conidia of *M. fructigena* and *M. cinerea*.

(1) On different plums of the same cluster.

Species.	Date.	Range of Variation.	Average.
<i>M. fructigena</i>	Sept. 18	12.0 × 9.0–27.5 × 15.0 μ	20.0 × 12.5 μ
<i>M. cinerea</i>	Sept. 17	8.5 × 6.5–22.5 × 18.0	17.0 × 10.5

(2) On opposite sides of the same plum, inoculated July 17.

Species.	Date.	Range of Variation.	Average.
<i>M. fructigena</i>	July 31	14.0 × 11.0–34.0 × 15.0 μ	21.0 × 13.5 μ
<i>M. cinerea</i>	"	10.0 × 7.5–27.0 × 14.5	16.0 × 11.0

It follows from these results that measurements of the conidia of *M. fructigena* and *M. cinerea* aid in diagnosing the species, but when the two are grown under the same conditions it is found that the difference is not so great as is generally supposed. The dimensions of the conidia as determined by continental workers are as follows:

Author.	<i>M. fructigena</i> .	<i>M. cinerea</i> .
Saccardo	25 × 10–12 μ	15–17 × 10–12 μ
Lindau	20–24 × 12–14	12–13 × 9–10
Woronin	Average 20.9 × 12.1	Average 12.1 × 8.8
Schröter	18–24 × 10–12	15–18 × 10–12
Aderhold and Ruhland	18–23 × 9–13	9.3–14.5 × 6.2–12.4

Woronin (27) found that when *M. cinerea* was growing on twigs or on the surface of various stone-fruits the average size of the conidia was 12.1 × 8.8 μ; the largest conidia he found under natural conditions measured 13.2 × 9.9 μ. It would seem from these figures that the dimensions quoted were those of conidia examined early in the year, and that the larger conidia produced on growing fruit in summer were not observed by Woronin; he found, however, that when *M. cinerea* was cultivated on artificially prepared media the average size of the conidia produced rose to 17.5 × 11.2 μ. Lindau and Aderhold and Ruhland also probably made their observations on conidia produced in winter, while Saccardo and Schröter must have examined summer conidia. This would explain the apparent discrepancies in the results obtained by the various workers.

In general terms the two species are to be distinguished by means of the conidia as follows:

*M. cinerea* produces in winter and early spring, on cankers, twigs, and mummied fruit, conidia the average length of which is about 11.5 μ,

while on recently infected fruit in summer the average length is about 17  $\mu$ .

*M. fructigena* produces conidia freely only in summer and autumn, and their average length is from 20  $\mu$  to 24.5  $\mu$ , according to the conditions under which they are produced.

(c) *Viability of the Conidia.*

During the winter months the pustules of *Monilia fructigena* are, as a rule, barren, as the conidia are mostly dispersed by the wind or washed away by rain in the autumn, and no others are produced until the approach of summer; those conidia still adhering to the pustules in winter are generally found to be collapsed or shrunken and incapable of germination, although particles of the pustules themselves, when placed in hanging drops of distilled water or on culture media, readily develop hyphae within 24 hours. These barren pustules differ in general appearance. Some are non-pulverulent; these vary in colour from a pale straw colour to almost white. Others are somewhat pulverulent or velutinous and are dark brown in colour; this condition is due to the presence of a hyphomycetous fungus with brown mycelium and conidia growing on the old pustules of *M. fructigena* (see Fig. 3).

On the other hand *M. cinerea* is stimulated to the production of pustules and conidia during winter. The form which produces the Blossom Wilt of apple trees, when it invades the tissues of the flowers, flowering spurs, and branches, may produce pustules on the pedicels and floral organs of the attacked flowers soon after infection, but this is not invariably the case, and, in so far as the author's experience has gone, appears to be the exception. Generally no pustules appear on the affected organs until about the beginning of December, when pustules begin to burst through the bark of the cankers and diseased spurs, and they continue to develop throughout the winter and spring. This condition, too, obtains in the young vegetative shoots of the plum when attacked by *M. cinerea*; conidia may be produced on the affected leaves, but pustules do not appear at the surface of the axis of the shoot until the approach of winter.

When *M. cinerea* occurs on fruit, e.g. plums and cherries, numerous pustules are produced during the summer (Pl. IV, Fig. 1), and, persisting on the mummified fruit, redevelop and become pulverulent during the following winter and spring (Fig. 2).

Apples which had been artificially inoculated with the apple Blossom Wilt form of *M. cinerea* in the laboratory produced no pustules, or very few, under those conditions, but when the infected black apples resulting from such inoculations were placed in the open air pustules burst through the skin and became covered with conidia during the winter.

Conidia produced at these low temperatures germinate readily when

placed in water. In January, 1917, during a period of severe frost, conidia of *M. cinerea* were taken from pustules growing on an apple tree in the open (the thermometer at the time showing a temperature of  $-2^{\circ}\text{C}.$ ) and mounted in a drop of water; the slide was placed in a damp chamber in the laboratory and within 24 hours 50 per cent. of the conidia had developed germ tubes.

These observations confirm conclusions arrived at by Ewert (9), who states that 'im Winter auf den Pflaumenmumien die Sporen der *M. cinerea* stets lebendig, die der *M. fructigena* stets tot sind'.

#### (d) Cultural Studies.

That *M. fructigena* and *M. cinerea* can be distinguished by their mode of growth on sterilized culture media has already been pointed out, and further study has confirmed previous observations. Prune juice agar has hitherto proved the best substrate for distinguishing the two species when growing vegetatively. On this medium, in Petri dishes, *M. fructigena* grows out uniformly to the edge of the plate in the form of an almost regular circular disc of mycelium (Fig. 6), while *M. cinerea* produces a more irregular growth with a tendency to form lobes (Fig. 5). Another characteristic of the latter species is its habit of zonate development; when the culture has made some growth (usually when it has reached about half-way to the edge of the plate, but often before this) further development is temporarily inhibited, but is soon renewed in the form of flabelliform outgrowths of mycelium which eventually coalesce to form a definite zone (Figs. 4 and 5). In some cultures three or four distinct zones are produced in this way.

In these plate cultures of *M. cinerea* f. *mali*, one or more dark brown zones invariably appear, the first at 5–10 mm. from the point of inoculation in about 12 days (Fig. 4); the brown zones are less regularly produced by the *Prunus* form and some strains remain quite hyaline (Fig. 5). This coloration is more pronounced in tube cultures, the zones becoming more or less confluent, so that in the case of the apple form the cultures may become almost black throughout.

Sterilized potato is another useful medium for diagnosing the species, particularly when they are found within the tissues of the host as barren mycelium only. On potato both species develop fructifications, each producing conidial tufts of the colour peculiar to the species, i. e. *M. fructigena* buff yellow pustules, *M. cinerea* grey ones. The two biologic forms of *M. cinerea* grow equally well on sterilized potato, but the conidial tufts of the apple form are generally less numerous and not so well developed as those of the *Prunus* form, and often some difficulty is experienced in obtaining sufficient conidia for inoculation experiments. On the other hand strains

which have been obtained from plums and cherries have always produced conidia freely on potato.

This difference in the conidial productivity of the two forms of *M. cinerea* has been noted also in the case of cultures growing on starch jelly prepared with the 'Modified Uschinsky's Solution' as recommended by Erwin Smith (24) for bacteria. Up to the present only two strains of each form have been cultivated on this medium in comparative tests. The two plum strains produced numerous conidia, but none could be found in the cultures of the two apple strains.

Cultural methods have also shown that there is a difference even in the mode of germination of the conidia when these are placed on prune juice agar. Typically the germ tube of *M. fructigena* grows out as a single hypha, almost straight, for some 400 to 1,200  $\mu$  before it branches to form a dendritic branching system terminal to the primary germ tube, within from 24 to 48 hours at room temperature; often a very short germ tube 10 to 30  $\mu$  in length develops at the opposite end of the conidium. The germ tube of *M. cinerea*, on the other hand, usually becomes geniculate and soon produces branches at a short distance from its point of origin, so that the branch system is very irregular from the first. In both species the hyphae, after 48 hours, grow out in all directions to form a more or less circular disc of mycelium.

When growing on agar culture media both *M. fructigena* and *M. cinerea* (including the American form of *Monilia*) produce numerous clusters of minute 'microconidia' or 'sporidia'. These spore-like bodies are globose and about 3  $\mu$  in diameter; attempts to induce them to germinate failed.

That cultural and biochemical methods employed in the laboratory will prove of service in distinguishing between morphologically similar forms of fungi is becoming more and more evident. Such methods are indispensable to the bacteriologist, and they offer to the mycologist a wide and interesting field for study. Alsberg and Black (2) found that biochemical methods were useful in identifying species of *Penicillium* which are not easily distinguished morphologically, and Grossenbacher and Duggar (12) were able to distinguish parasitic and saprophytic strains of *Botryosphaeria ribis* by means of artificially prepared cultures.

#### (e) *American Strains of Monilia.*

Until a few years ago plant pathologists in North America had attributed Brown Rot diseases in that continent to *Monilia fructigena*. Recent writers, however, conclude that it is identical with *Monilia cinerea*, Bon.: thus Bartram (3) writes: 'The common form of brown rot of stone fruits, as found in Vermont, is due to the fungus known in Europe as *Sclerotinia cinerea*. This is conclusively proved not only by the measurements of the conidia, the absence of disjunctors, the grey colour of the conidial tufts, but more

especially by the persistence of the vitality of the conidia through the winter.'

Ten strains of this American form have been received at Wye from three different localities in North America, viz. Wisconsin, Oregon, and Ontario, and from three different hosts, viz. apple, plum, and peach. They have been cultivated and examined side by side with cultures of the forms occurring in this country. It was found that the American *Monilia* could be distinguished in cultures from the forms of *M. cinerea* occurring in England, in the readiness with which it produces conidia, and in its mode of growth when cultivated on prune juice agar.

The European form does not produce conidia<sup>1</sup> on prune juice agar or carrot agar, nor in such liquid media as fruit extracts or Coons' solution, while the American strains develop numerous well-developed pustules on all these media. On prune juice agar the American form grows out uniformly to the edge of the plate, and in this respect resembles *M. fructigena* rather than our native forms of *M. cinerea*; these cultures, however, can easily be distinguished from those of *M. fructigena* by the numerous small grey tufts of conidiophores which develop (Fig. 7).

Again, in the mode of branching of the germ tube of the conidium, on agar plates, the American form of *Monilia* more nearly resembles *M. fructigena* than *M. cinerea*. The germ tube shows less tendency to become geniculate than is the case with that of *M. cinerea*; it is usually at least 200  $\mu$  in length before it begins to branch, and unbranched germ tubes 650  $\mu$  and 750  $\mu$  in length have been observed.

#### (f) *Taxonomy.*

The results of observations and experiments recorded in the present paper show that there are two distinct species of *Monilia* occurring in Britain, and that they are to be distinguished as follows:

	<i>M. fructigena.</i>	<i>M. cinerea.</i>
Pustules.	Buff yellow.	Grey, generally smaller than those of <i>M. fructigena</i> .
Dimensions of conidia.	Average about $21 \times 13 \mu$ .	Approx. average. Winter conidia, $11.5 \times 8 \mu$ . Summer conidia, $17 \times 11 \mu$ .
Mode of germination of conidia on prune juice agar.	Typically produces a long germ tube, 600 to 1,200 $\mu$ in length, before branching.	Germ tube branches while still quite short, often close to its point of origin; it is usually geniculate at one or more points.
Cultures on prune juice agar plates.	Growth uniform to edge of plate; margin entire or sub-entire.	Growth zonate; margin lobed.
Winter condition.	Pustules generally barren in winter or bearing non-viable conidia.	Pustules and conidia produced freely in winter.

<sup>1</sup> A few conidia tufts have been observed in one instance only when prune juice agar was used, in a tube culture of a plum strain which had been growing at a fairly low temperature ( $7^{\circ}$ – $15^{\circ}$  C.) for some weeks.

The biological relationship of the two species to their host is also different. Observations made in Kent on affected trees, and on specimens received from other counties, show that the species occur on apples, plums, and cherries as shown in the following table. Observations on other hosts have not been sufficiently numerous for a general statement to be made of their mode of attack.

Host.	<i>M. fructigena.</i>	<i>M. cinerea.</i>
Apple trees.	Produces a fruit rot and may cause cankers by invading the branches from the fruit.	Produces a blossom wilt and often causes cankers by invading the branches through the flowers and flowering spurs.
Plum trees.	Causes a fruit rot.	Produces blossom wilt, cankers, and fruit rot.
Cherry trees.	Causes a fruit rot.	Produces blossom wilt and may kill twigs; it also causes a fruit rot.

Not only are there two distinct species of *Monilia* concerned in the Brown Rot diseases, but there is evidence, as presented in the preceding pages, that in Britain there are two biologic forms of *M. cinerea*, which may conveniently be referred to as forma *mali* and forma *pruni*; these may be distinguished as follows:

<i>Monilia cinerea</i> forma <i>mali</i> .	<i>Monilia cinerea</i> forma <i>pruni</i> .
Produces a blossom wilt and canker of apple trees.	Unable to cause a blossom wilt of apple trees.
Readily secretes an enzyme which oxidizes tannins.	Secretes the oxidizing enzyme far less freely.

*M. cinerea* forma *mali* appears to be confined to the apple; I have not yet found it on plums or cherries, although artificial inoculations have shown that it is able to infect the flowers and fruit of the plum tree.

*M. cinerea* forma *pruni* is the form occurring on plums, cherries, and damsons; it is found also on *Pyrus japonica*. The fact that it is unable to invade the flowering axis of the apple tree to cause a blossom wilt accounts for its inability to establish itself on that host.

A comparison of the morphological and cultural characters of strains of *M. fructigena* and *M. cinerea* isolated from mummied fruit sent by Dr. Quanjér from Holland showed that the two species occurring in this country are similar to those of the Continent. I have not yet been able to secure specimens of *M. cinerea* f. *mali* from abroad, but the description and illustrations given by Eriksson (8) of a disease of apple trees occurring in Sweden conform so closely with the appearance of the 'Blossom Wilt and Canker' disease of apple trees occurring in this country that there is good reason for assuming its occurrence on the Continent.

There is no record of the occurrence in Britain of the ascigerous stage in the life-history of either *Monilia cinerea* or *M. fructigena*, and all attempts at Wye to induce the development of apothecia by placing mummied fruit on the ground in the open have hitherto failed.

The *Sclerotinia* stage of *M. fructigena* was first described by Aderhold and Ruhland (1), the genetic connexion between the ascigerous and the conidial forms being established by the development of the *Monilia* form from ascospores.

The same workers were also able to obtain a *Sclerotinia* from mummied apricots, and from the ascospores obtained cultures which developed conidial pustules resembling those of *M. cinerea*; this Ascomycete they named *Sclerotinia laxa*, i.e. the ascigerous stage of Ehrenberg's *Oidium laxum* (= *Monilia laxa*, Sacc. et Vogl.). They considered *Monilia laxa* to be distinct from *M. cinerea*, morphologically with regard to the size of its conidia, and biologically from the fact that they found it occurring plentifully on apricots when neighbouring peaches and cherries were unaffected. The size of the conidia of *M. laxa* Aderhold and Ruhland give as  $12.4-23.8 \times 9.3-15.5 \mu$ , the dimensions of those of *M. cinerea* as  $9.3-14.5 \times 6.2-12.4 \mu$ . It is to be observed, however, that the dimensions given for *M. laxa* conform to those of *M. cinerea* when the latter is growing in summer on plums.

To the time of writing, only one strain from apricots has been examined at Wye; this was found on dead twigs in February 1919. The size of the conidia was found to be from  $6.5 \times 3 \mu$  to  $14.5 \times 9.5 \mu$  with an average of  $11 \times 7.5 \mu$  for 100 conidia, dimensions which are of the same order as those of the winter conidia of *M. cinerea* produced on mummied plums and cherries. There seems to be no valid reason, therefore, for considering the two conidial forms *M. cinerea* and *M. laxa* as two distinct morphological species; whether the apricot has a biologic form confined to that host remains to be proved by inoculation experiments with pure cultures.

In North America a Brown Rot *Sclerotinia*, first discovered and described by Norton (20), is frequently found. Specimens, preserved in spirit, were sent by Norton himself to Aderhold and Ruhland, and they named it *Sclerotinia cinerea*, assuming it to be the ascigerous stage of the conidial form *Monilia cinerea*, Bon. Since, as shown in this article, the American form of *Monilia* is readily distinguished in cultures from the *M. cinerea* of Europe, the writer regards it as a distinct form (provisionally referring to it as *Monilia cinerea* forma *americana*) of which the *Sclerotinia* stage is that recently described from fresh material by Matheny (18). Whether this *Sclerotinia* of America will prove to be identical with, or distinct from, the ascigerous stage of the European form *Monilia cinerea*, Bon., must remain an open question until an Ascomycete is described the ascospores of which give rise to a *Monilia* morphologically and culturally identical with that occurring on *Prunus* spp. in Europe.

As there are reasons for suspecting that *Monilia laxa* and *M. cinerea* are the same morphological species, it appears probable that *Sclerotinia laxa* (Ehrenb.), Aderh. et Ruh., is the ascigerous stage of both the conidial forms



described under those names by various authors, but until this relationship is established experimentally the present writer prefers to retain the name *Monilia cinerea*, Bon., for the grey *Monilia* occurring parasitically in Europe on the commonly cultivated species of fruit trees of the genera *Pyrus* and *Prunus*.

# SUMMARY.

(1) In Britain there are two species of *Monilia* parasitic on fruit trees of the genera *Pyrus* and *Prunus*, viz. *M. fructigena*, Pers. = *Sclerotinia fructigena* (Pers.), Schröt., and *M. cinerea*, Bon.

(2) The two species are to be distinguished by—

- (a) the colour and size of the pustules,
- (b) dimensions of the conidia,
- (c) mode of growth on sterilized culture media,
- (d) mode of branching of germ tube of conidium,
- (e) mode of parasitism.

(3) *M. fructigena* causes a fruit rot of apples, plums, and cherries, and on apple trees may produce cankers by invading the branch through the fruit.

*M. cinerea* occurs on apple, plum, and cherry trees and causes diseases as follows:

Apple trees—'Blossom Wilt and Canker' disease.

Plum trees—fruit rot, blossom wilt, cankers, and a 'Wither Tip' disease of young shoots.

Cherry trees—fruit rot, blossom wilt, and may kill twigs by entering through the flowers.

(4) In *M. fructigena* conidia production is inhibited during winter; *M. cinerea*, on the other hand, begins to develop new pustules about the beginning of December and produces conidia freely during winter and spring.

(5) The winter conidia of *M. cinerea* are considerably smaller than those produced by it in summer.

(6) The form of *M. cinerea* parasitic on apple trees is biologically and physiologically different from the form found on plum and cherry trees:

*M. cinerea* forma *mali* produces a blossom wilt of apple trees; it readily secretes an enzyme which oxidizes tannins.

*M. cinerea* forma *pruni*, when apple flowers are inoculated with conidia, attacks only the flowers actually inoculated and does not invade the axis of the inflorescence; it secretes the oxidizing enzyme far less freely than the apple form.

(7) The oxidizing enzyme is secreted freely by *M. cinerea* f. *mali* when growing in—

- (a) liquid culture media,
- (b) infected apples,
- (c) infected apple spurs.

(8) The American form of *Monilia* is more nearly related to *M. cinerea* than to *M. fructigena*, but in cultures it can be distinguished from the European form of *M. cinerea* by its mode of growth in cultures and by the numerous fructifications it produces on all the culture media on which it has been grown.

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## EXPLANATION OF PLATES IV AND V.

Illustrating Mr. Wormald's paper on the 'Brown Rot' Diseases of Fruit-trees. II.

Fig. 1. Plums affected by Brown Rot caused by *Monilia cinerea*, showing—

(a) infection arising from contagion;

(b) the small grey pustules typical of the fungus when growing on recently infected fruit.

Fig. 2. The winter condition of plums infected with *M. cinerea*; the redeveloping pustules are larger than those produced in summer.

Fig. 3. The winter condition of plums infected with *M. fructigena*; the pale pustules are smooth (non-pulverulent) and barren; the darker pustules below and on the left are covered with a brown hyphomycete.

Fig. 4. *M. cinerea* f. *mali*

Fig. 5. *M. cinerea* f. *pruni*

Fig. 6. *M. fructigena*

Fig. 7. American strain of *Monilia*

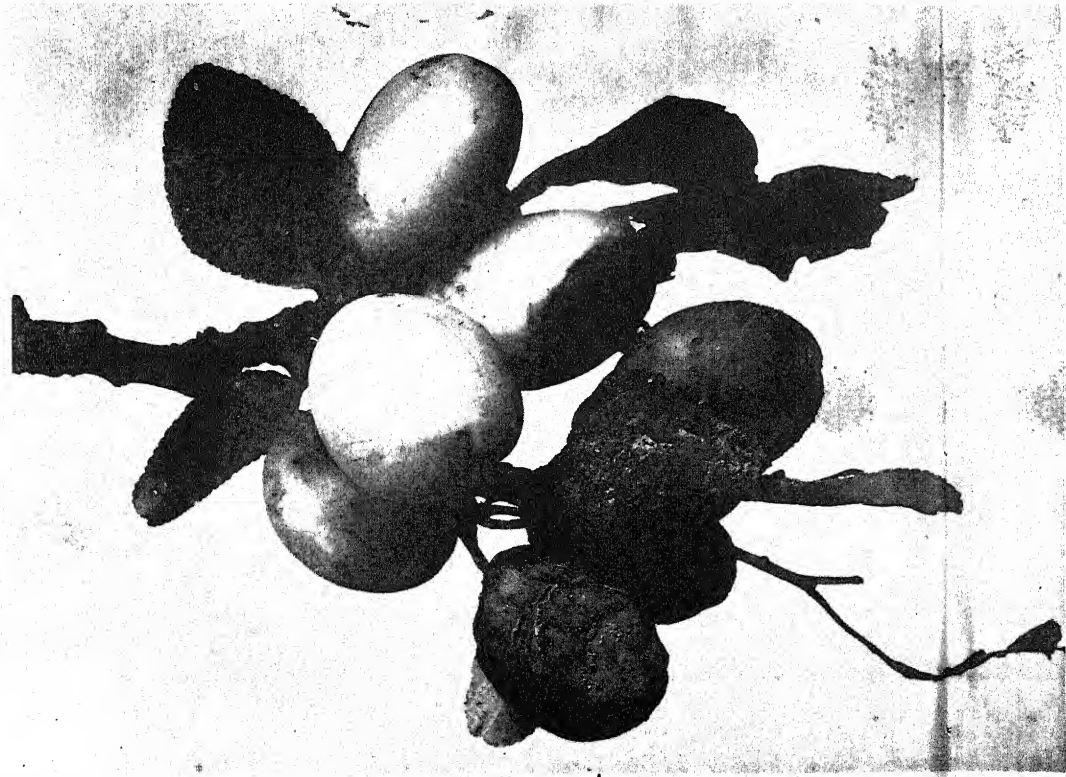
Cultures of the same age grown simultaneously under the same conditions, i.e. on prune juice agar at room temperature (about 16° C.). The granular appearance in Fig. 7 is due to innumerable tufts of conidiophores with chains of conidia.

Fig. 8. The relative colour reactions with guaiacum shown by three extracts, similarly prepared, from the tissues of one and the same apple which had been artificially inoculated with two strains of *M. cinerea*:

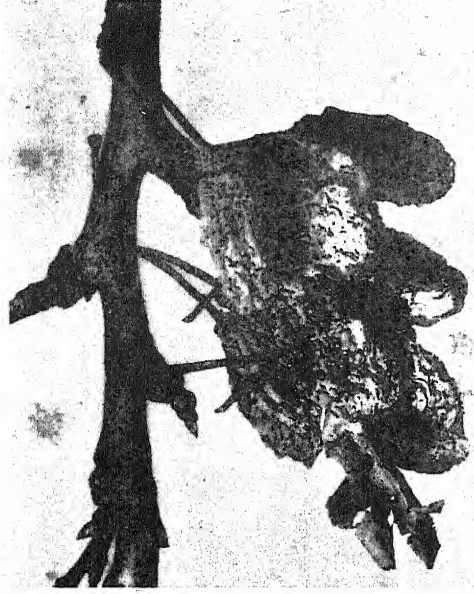
A. From side infected with *M. cinerea* f. *mali*.

B. " " " " *M. cinerea* f. *pruni*.

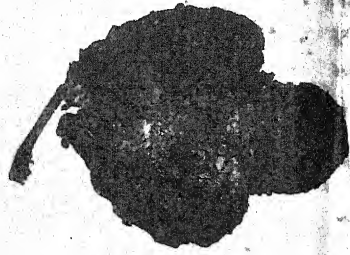
C. From that portion of the apple which had not become infected.



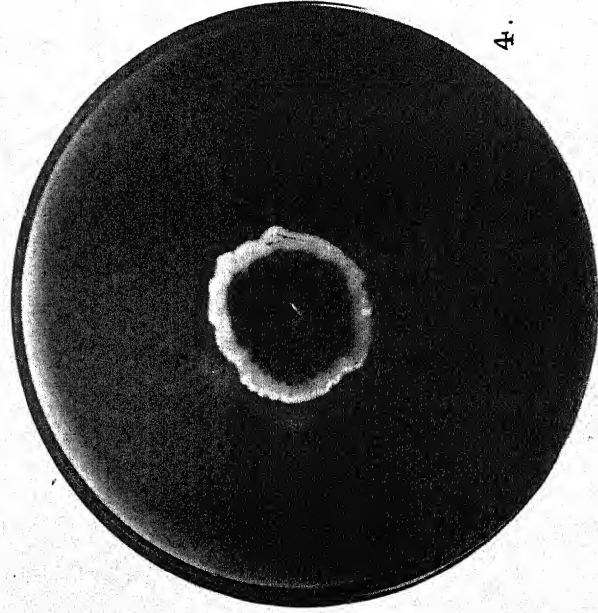
1.



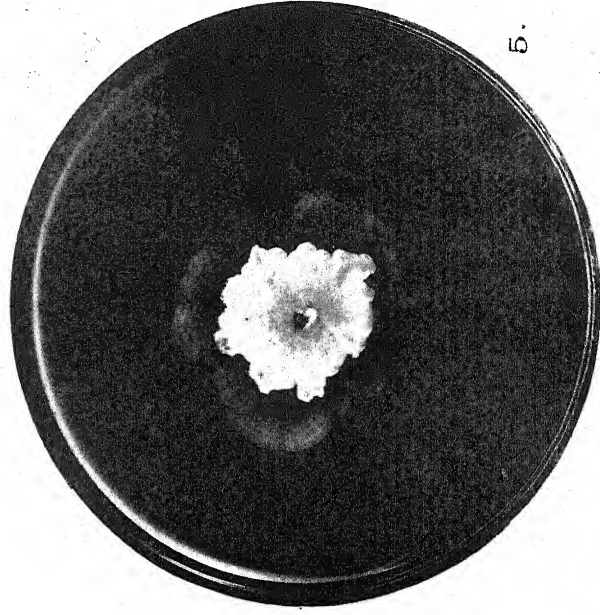
2.



3.



4.

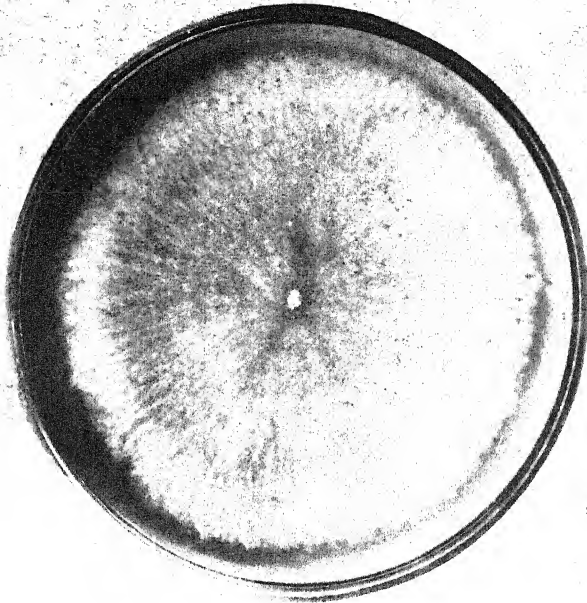


5.

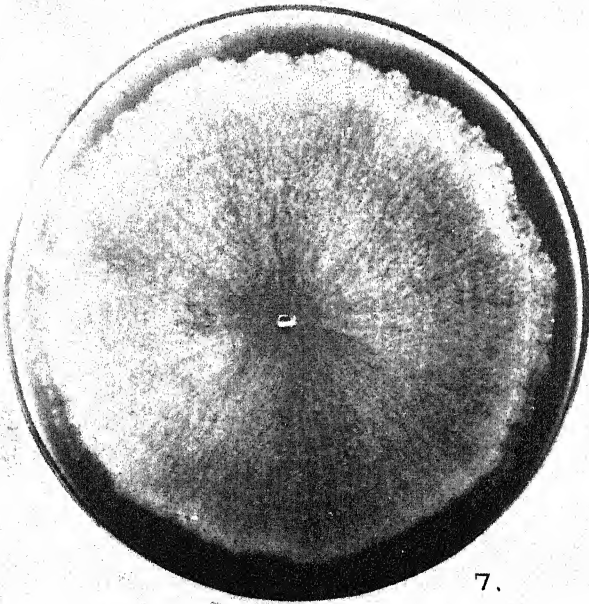
WORMALD — BROWN ROT.

Huth, coll



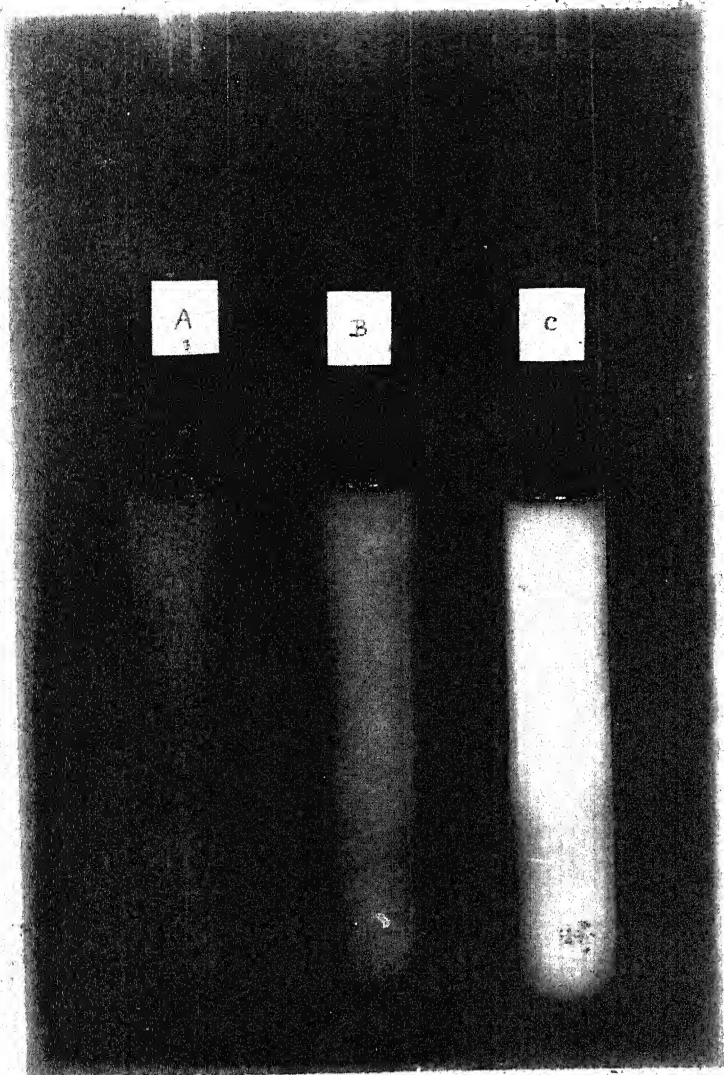


6.



7.

WORMALD — BROWN ROT.



8.

Huth, coll.

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# Puccinia malvacearum and the Mycoplasm Theory.

BY

M. A. BAILEY, B.A., Cantab.

With two Figures in the Text.

## INTRODUCTION.

THE sudden and widespread appearance, year after year, of certain parasitic fungi is a phenomenon which has been recognized for many years and has given rise to the keenest controversy and speculation.

The mode of origin of these epidemics is very difficult to discover, and the question is rendered more complex owing to the fact that the fungi concerned are often extremely narrowly specialized and that their spores frequently show a very limited period of viability.

After a lengthy series of experiments on the Rusts of various species of Gramineae, Eriksson brought forward the Mycoplasm Hypothesis in explanation of the observed facts.

This hypothesis was first asserted by him in 1897, but has been developed and enlarged upon subsequently to include other fungi in addition to the Cereal Rusts.

In 1911 Eriksson published a monograph on *Puccinia malvacearum*,<sup>1</sup> in which he applied this theory to account for the yearly origin and dissemination of the Hollyhock Rust, and more recently a similar explanation has been put forward by him in the case of the Potato Disease Fungus, *Phytophthora infestans*.<sup>2</sup> Briefly stated, his suggestion is that the fungus, in certain cases, is able to spend part of its vegetative life in the form of naked protoplasm in the interior of the cells of the host, existing there in a symbiotic state.

When in this condition the fungus is said to have assumed the mycoplasmic state, and this mycoplasm permeates the whole of the plant tissues, including the developing embryo.

<sup>1</sup> Eriksson : Der Malvenrost ; seine Verbreitung, Natur und Entwicklungsgeschichte. Kungl. Svenska Vetenskapsakademiens Handlingar, Band xlvii, No. 2.

<sup>2</sup> Eriksson : Développement primaire du mildiou (*Phytophthora infestans*) de la pomme de terre. Rev. Gén. de Botanique, tomes xxix and xxx.



When the seed germinates in the following season, the fungus protoplasm divides with the cytoplasm of the host cells and is in this way distributed throughout the tissues of the seedling plant.

Later on, when the growing plant reaches a certain stage of maturity, the fungus reverts to the mycelial condition preparatory to the production of spores.

The theory is exceedingly attractive and is backed by a mass of data derived from experimental inoculation and histology. If proved correct, it would have most far-reaching effects in modifying the theory and practice of the control of plant disease.

Eriksson's hypothesis has, however, many opponents, and certain isolated experimental results, recorded by other investigators, suggest that the facts can be explained by other and simpler means.

It was with the idea of throwing further light on this subject that the experiments described in the present paper were started.

The experiments were interrupted by the war, and there seems, unfortunately, to be little chance of repeating them in the near future, but the evidence obtained is, in many cases, in such marked opposition to the observations and deductions made by Eriksson as to seem to warrant publication in some detail.

The work was carried out at the John Innes Horticultural Institution, where I was given every facility through the kindness of Mr. Bateson.

I should like to take this opportunity of thanking Mrs. Scott Tucker and Miss D. M. Cayley for great assistance rendered in the recording of results during the later stages of the experiments.

An extensive bibliography is considered to be unnecessary in this paper, as a list of the more important literature will be found at the end of Eriksson's paper on 'Der Malvenrost', already referred to.

#### DISCUSSION OF ERIKSSON'S WORK ON THE HOLLYHOCK RUST.

In his paper on the Hollyhock Rust, Eriksson emphasizes a number of points which he considers as affording strong evidence of the existence of a mycoplasmic stage in the life-history of the fungus. These points are enumerated below, where they are considered in the light of further evidence.

1. *Sudden appearance of the disease in epidemic form.* This has long been recognized by many observers. Eriksson states that the interval between the sowing of the seed and the appearance of the disease in quantity is extremely regular and varies only with the time of year. Seed sown in Sweden at the end of June produced plants on which the rust appeared regularly about three months later. On no occasion did the plants show rust earlier than three months after germination, except in those cases where they were grown close to other plants which were already suffering from the disease.

Seed sown in September germinated the same year, and the young plants, if kept in boxes in a cold house during the winter, did not show any trace of disease till the following May.

2. *Limitation of disease outbreak to leaves of a certain age.* 'Primary' and 'secondary' development. Eriksson calls attention to the fact that the leaves of affected plants show little or no infection whilst young, but when they arrive at a stage approaching maturity they become densely covered with pustules of the rust. Leaves that escape infection at this time never seem to become heavily infected.

The conclusion that Eriksson draws from these observations is that the sudden appearance of great quantities of pustules on the middle-aged leaves represents the separation of the mycoplasma existing within the leaf tissues and its differentiation into vegetative and reproductive mycelium. The sporadically distributed pustules appearing on leaves of various ages he attributes to outside infection. These observations have been confirmed in part by the present writer, but the conclusion drawn is that the metabolism of the middle-aged leaf is such as to render it much more susceptible to attack than younger or older leaves. This conclusion is supported by observations made in the present season on the incidence of rust upon wild plants of *Malva sylvestris*.

About a dozen plants altogether were found and marked for future record. These will be referred to by letters as shown below:

'A.' Four plants growing within a radius of 8 feet of one another.

'B.' Two plants growing close together at a place half a mile distant from 'A'.

'C.' Two plants close together about half-way between 'A' and 'B'.

'D.' Five plants scattered at long intervals over an area situated almost a mile from any of the above.

When first observed, on the 14th of May, one of the 'A' plants showed a few young pustules on four leaves (one of which was still quite young).

One leaf of one of the 'C' plants was similarly affected.

On May 20 all the middle-aged leaves of the plant of the 'A' group mentioned above were affected, carrying an average of 30 pustules apiece. Some of the younger leaves of the same plant also showed a few pustules. The plant nearest this one in the same group showed a few pustules sporadically distributed. The infected plant in the 'C' group showed a slight general increase of disease. None of the other plants showed disease.

On May 26 the first infected plant in 'A' group showed dense masses of pustules on all its middle-aged leaves and sporadic infection on most of the younger and some of the older leaves. The plant next to it was in practically the same condition, and both answered the description of 'primary infection' given in Eriksson's paper. Of the other two plants in

group 'A' one showed slight infection of the mature leaves and the other one showed a few pustules only. The plant in group 'C' previously recorded as slightly infected now showed a degree of infection corresponding to rather heavy 'secondary infection' in Eriksson's description. One of the plants in group 'B' and one of the isolated plants referred to in the original description under the letter 'D' now showed one or two pustules on middle-aged vigorous leaves. The above account summarizes the appearance of these plants on the dates given: seen from day to day the impression was even more forcibly conveyed that this was a case of disease spreading from one plant to another by means of a normal process of infection, either from the plants originally recorded as diseased, or from others in the vicinity, which had been overlooked.

The transition in the case of the two heavily infected plants in group 'A' from a state of moderate infection of 'secondary' type to that resembling very heavy 'primary infection' was rapid, but showed all intermediate stages. All these plants arose from perennial root stocks, and in the case of group 'A' they grew in a yard which was known to have contained heavily diseased plants of *Malva sylvestris* for several years previously.

3. *Sudden appearance of disease in bulk in places far removed from outside sources of infection.* The facts given by Eriksson are remarkable, but the observations just recorded above show that in cases of 'normal' infection the infective material can be and often is carried long distances, and that the appearance of the first few isolated pustules is followed very rapidly by conditions of intense infection. Moreover, these records show the existence of a relatively large amount of infective material, in this country at least, at an early period in the season of growth.

4. *Non-viability of teleutospores which have passed through the winter without germinating.* It is an established fact that the vast majority of over-wintered spores of this fungus lose their power of germination. Eriksson quotes the work of previous investigators in support of this, and records a few cases in which he himself tried to germinate such spores, but without success. The experience of the present author points in the same direction. Eriksson, however, quotes Fischer as finding germinable teleutospores as far through the winter as January 31, and Taubenhaus<sup>1</sup> in America has shown that such spores can be germinated as late as April in the year following their formation. The last named points out that the older the spore is the longer it takes to germinate, and suggests that previous negative results might likely be explained on the assumption that the teleutospores had not been left long enough in the drops of water.

That this delayed germination takes place but sparingly in nature is to

<sup>1</sup> Taubenhaus: *Puccinia malvacearum*, Mont. Phytopathology, vol. i, No. 2.

be inferred from an experiment conducted by Dandeno,<sup>1</sup> who scattered a large quantity of dead leaves and stems of mallows, which had been diseased the previous autumn, amongst patches of mallows which were just commencing to grow in the following spring. The infected litter produced no result.

5. *Absence of mycelium in seeds and young plants.* Eriksson made numerous sections of both seeds and young plants, and failed in every case to find any trace of mycelium.

Though it may be taken as an established fact that the fungus does not pass through the winter in a mycelial condition in the seed, there is evidence to suggest that in certain cases the mycelium persists in the stems and leaves of hollyhock or mallow plants growing in positions where they are sheltered by surrounding vegetation or litter. Taubenhaus<sup>2</sup> records a case in which he found living mycelium and even immature teleuto-sori throughout the winter and early spring in hollyhock leaves which had been mulched with horse manure.

It may easily be imagined that the comparatively few spores which are needed to start the initial infection are derived chiefly from such persistent mycelium, though a certain proportion of spores doubtless survive the winter and germinate direct in the following spring.

6. *Dimorphism in teleutospores.* Eriksson found that teleutospores differed in the mode of their germination. Either a short thick septate promycelium is formed, which abstricts small pear-shaped sporidia from each of its segments, or a comparatively long thin promycelial tube may arise, which is marked by the fact that its base is frequently somewhat swollen and that it never produces sporidia.

In this latter case the distal end of the tube divides up into three or four oblong segments which ultimately fall apart in a way which strongly recalls the formation of oidia in the Erysiphaceae.

Taubenhaus also describes the formation of these 'oidia', and states that they subsequently germinate to form sporidia, which in turn germinate by the production of germ tubes.

The present writer has been unable to confirm this last observation of Taubenhaus, but has frequently seen 'oidia' germinating *direct* by means of germ tubes.

Eriksson lays very great stress on this dimorphism in promycelia, though he admits that the two forms of germination may sometimes be found in the same pustule. He states that spores taken in spring from pustules on plants which have been wintered over in greenhouses—and *in* which the fungus has consequently continued to live—germinate mostly

<sup>1</sup> Dandeno: Life-history of *Puccinia malvacearum*. Ninth Annual Report Mich. Acad. Sci., 1907.

<sup>2</sup> Loc. cit.

with the production of promycelia and sporidia; sometimes 'oidial' germination may be found to occur in the same pustule, and occasionally all the germination in any one pustule will be of the oidial type. On the other hand, he says, spores taken from pustules which had broken out on plants which had passed the winter out of doors and had lost their leaves in the normal way, germinate almost always with 'oidia'.

These conclusions are supported by a long table containing the results of 34 experiments, but the evidence is not very convincing owing to the frequent occurrence of both sorts of germination from one pustule and to the fact that in certain cases one pustule has given entirely sporidial germination, whilst another from the same plant has given entirely oidial germination.

The present writer has conducted a fairly extensive series of germination experiments, the details of which will be found in the Appendix to this paper.

The deduction made from these experiments is that the 'oidial' type of germination is merely the result of abnormal conditions and can be produced at will by varying the environment.

If the teleutospores are completely submerged in water, germination will be almost exclusively of the 'oidial' type, whilst the same spores, exposed to moist air, will produce promycelia and sporidia in the normal way.

The experiments are divided into six series. In the first three series, spores, teased apart or united in pustules, were placed either on or under the surface of water in watch-glasses. It may be assumed in this case that all those spores which remained at the bottom of the water were thoroughly wetted, whilst those which floated on the surface were either partially or wholly wet or, less frequently, quite dry.

An examination of the results of these three series shows that spores which remained at the bottom of the water germinated exclusively in the 'oidial' manner, whilst those at the surface germinated in either one way or the other. In almost all cases the spores on the surface showed a mixed germination, and in a few cases the two cells of the one spore germinated differently. One such case is illustrated in Fig. 1.

Eriksson, in his description of the oidial type of germination, calls attention to the swelling at the proximal end of the germ tube. In our own cultures this swelling has frequently been noticed, but is by no means an invariable feature of this type.

In Series IV and V complete leaves were detached from diseased plants and supported in such a way that one half of each leaf was submerged in water whilst the other half was exposed to the air.

The air around the leaves was kept thoroughly moist by covering the whole with a large bell-jar. Series VI was carried out similarly with

detached leaves, but in this case one leaf was entirely submerged, whilst another similar leaf from the same plant was kept in moist air. The results of these three series were uniform in one respect: in every case the pustules which were exposed to the air germinated to form promycelia and sporidia in the normal manner. The behaviour of the submerged leaves or portions of leaves was not so regular; sometimes oidia only were produced, some-

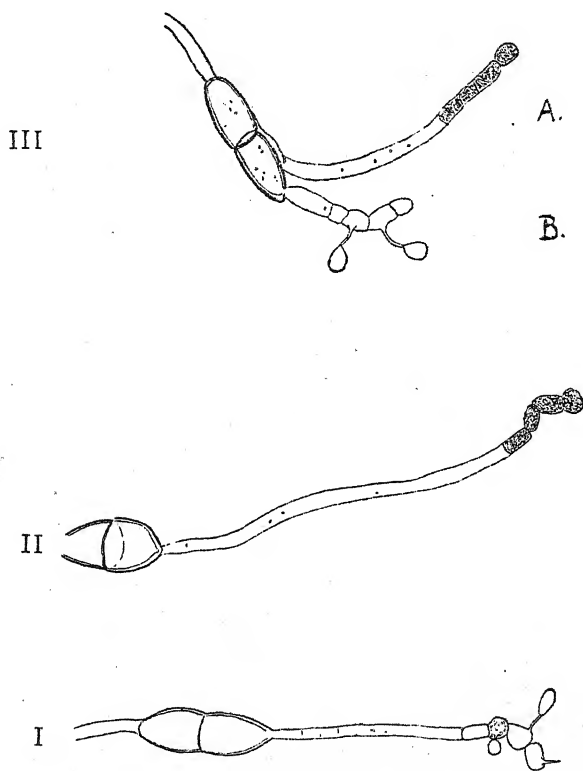


FIG. 1. Teliospores of *Puccinia malvacearum* germinated in sterile tap-water in a watch-glass.  
 I. Shows typical sporidial germination of a spore lying on surface of water.  
 II. 'Oidial' germination of a spore at bottom of watch-glass.  
 III. Shows 'oidial' germination (A) from the proximal cell (whole of germ tube under surface of water) and sporidial germination (B) from distal cell (the three last segments of the germ tube are above the surface of the water).  
 (Magnification, 380 diameters.)

times a mixture of oidia and abortive germ tubes, more or less intermediate between the two types, and sometimes, but much more rarely, a normal crop of sporidia was formed.

These apparent irregularities are without doubt due to one of two causes: either the whole surface of the leaf was not in every case thoroughly wetted, or the pustules had already started to germinate before they were submerged. That this is the correct interpretation cannot be doubted when

the absolute regularity of behaviour of the submerged spores in Series I to III (17 cases) and of the exposed spores in the remaining series (93 pustules) is taken into account.

An alternative explanation is that the pustules contain spores which can only germinate in one way or the other, and that sporidial germination is inhibited by very wet conditions and vice versa.

This, however, is very difficult to reconcile with the fact that the two cells of a single spore, floating on the surface of water, sometimes germinate differently. Nor is it supported by the evidence as to the proportion of germinated to ungerminated spores in the two kinds of cultures: germination was very irregular throughout, and no connexion can be traced between full and scanty germination and the production of one particular kind of spore.

A noticeable feature in the behaviour of the oidial spores was their tendency to early disorganization, but this may have been due merely to the fact that the bacteria, which caused their decay, were unduly favoured by the presence of the surrounding water.

7. *Difference in behaviour of spores in inoculation experiments.* Eriksson gives results in tabular form showing that infection experiments with those teleutospores which germinate to form sporidia almost always give positive results, whilst teleutospores which produce oidia give apparently negative results. In one case he got a positive result by infecting with a pustule of oidia-forming teleutospores, but this he explains by assuming that there were a certain number of sporidia mixed up with the other spores.

If the view is accepted that the oidial form of spore is the result of abnormally wet conditions, it would seem likely that the spore, being itself abnormal, would show a decreased infective power.

This would appear to have been the case in Eriksson's investigations, but the experiments conducted by the present author show no particular prepotence of either of the two forms.

The results of these experiments, which were relatively few in number, are given in tabular form below. They were carried out during a spell of very hot weather under conditions which were not very favourable to the fungus.

The method used was that described by Eriksson in his paper. Each leaf was inoculated in six places—three on either side of the midrib. The inoculations on the right side of the midrib were carried out with pustules which had germinated to form sporidia only, whilst those on the left side were carried out with pustules which had produced oidia only.

The plants used were hollyhocks, bearing about twelve leaves apiece and just beginning to send up flowering spikes. Inoculations were made on the upper surface of the leaves.

One leaf only of each plant was inoculated in the manner described,



the rest being kept as controls. No rust appeared on any of the uninoculated leaves during the experiment.

One pustule was used for each inoculation, and these were taken from leaves which had been kept for some time previously either submerged in water or exposed to a damp atmosphere.

The mode of germination of the teleutospores was ascertained by microscopical examination before the pustules were employed for purposes of inoculation.

TABLE I.

Date.	Plant No.	Inoculation Number.	Material used for inoculation.		Results.
			Nature.	Condition.	
3.6.19.	1	1	sporidial germination	good	6 pustules on lower surface.
		2	" "	"	1 pustule on lower surface.
		3	" "	"	Failed.
		4	oidial "	} oidia showed tendency to disorganize rapidly	"
		5	" "		"
		6	" "		"
		2	sporidial "	good	No pustules, but several small black flecks of dead tissue, showing localized lethal effects of the fungus.
		8	" "	"	Ditto
		9	" "	"	Failed—no black flecks.
		10	oidial "	} oidia showed tendency to disorganize rapidly	Failed.
4.6.19.	3	11	" "		"
		12	" "		"
		13	sporidial "	good	3 pustules on lower surface.
		14	" "	"	1 pustules on lower surface.
		15	" "	"	1 pustule on lower surface and considerable number of black flecks.
		16	oidial "	"	13 pustules on lower surface.
		17	" "	"	4 pustules on lower surface.
		18	" "	"	1 pustule and considerable number of black flecks.
		4	19 sporidial "	"	Failed.
			20 " "	"	"
			21 " "	"	"
			22 oidial "	"	"
			23 " "	}	Failed, but fair number of black flecks.
			24 " "		
		5	25 sporidial "	"	1 pustule on lower surface.
			26 " "	"	2 pustules on lower surface and few black flecks.

TABLE I (continued)

Date.	Plant No.	Inoculation Number.	Material used for Inoculation.		Results.
			Nature.	Condition.	
4.6.19.	5	27	sporidial germination	good	Failed.
		28	oidial "	"	2 pustules on lower surface and several black flecks.
		29	" "	"	1 pustule on lower surface.
	6	30	" "	"	Failed.
		31	sporidial "	"	"
		32	" "	"	Failed, but showed a few black flecks
		33	" "	"	Failed.
		34	oidial "	"	Failed, but considerable number of black flecks.
		35	" "	"	2 pustules on lower surface, and considerable number of black flecks.
		36	" "	"	2 pustules on lower surface and considerable number of black flecks.
	7	37	sporidial "	"	1 pustule on lower surface. Many black flecks.
		38	" "	"	Failed, but many black flecks.
		39	" "	"	Ditto.
		40	oidial "	"	Ditto.
		41	" "	"	Ditto.
		42	" "	"	1 pustule on lower surface. Many black flecks.

The occurrence of the black flecks of dead tissue in the inoculated areas of the leaves is interesting as showing the local lethal effects that the spores are capable of producing. They may occur with either sort of spore, and may possibly be connected with the histological changes in the cells of the leaf, which Eriksson has described in cases of negative infection, and which he regards as the visible signs of mycoplasmic entry.

8. *Cytological changes in case of 'negative' infections.* Eriksson describes certain changes in the protoplasm of some of the epidermal cells, which he considers are due to the penetration of the protoplasm of 'oidial' spores, lying outside, into the cavity of the epidermal cells in the form of mycoplasma.

Eriksson himself states that the channels by which the mycoplasma enters are too fine to be seen, and also admits that cases, similar to that just described, also occur side by side with normal positive infections.

In view of this and of the lack of circumstantial evidence, it would seem simpler to explain the changes he describes as local lethal effects due

to the ungerminated spores. It may even be suggested that the spores do not germinate *because* they have formed within themselves some substance which is toxic to themselves, and that this same substance, on escaping, reacts even more noticeably on the cells of the plant underneath.

9. *Histological distinction between growing seedlings derived from clean and 'infected' seed.* The appearances described by Eriksson are similar to those which he had previously described in the case of wheat, and are open to the same criticisms which Marshall Ward<sup>1</sup> raised at that time.

The present author has not had the opportunity of repeating this histological work, but has attempted to prove the presence or otherwise of the fungus in the tissues by growing plants from seed onwards under conditions which afforded protection from outside infection.

The manner in which these experiments were carried out and their results are shown in the next section of this paper.

#### EXPERIMENTS IN GROWING HOLLYHOCK PLANTS FROM SEED UNDER CONDITIONS EXCLUDING OUTSIDE INFECTION.

Dandeno, in the paper which has already been referred to, makes a record to the effect that he collected and planted many affected seeds, which were 'kept under conditions unfavourable to inoculation from external sources', and that in no case did rust appear on these plants. He makes no reference to the manner in which the experiment was conducted nor any reference to controls.

During the years 1912 to 1915 an attempt was made by the present writer to carry out a series of cultures under critical conditions. Two experiments were carried out during that time and will be described separately below.

##### *Experiment 1.*

The essential part of the apparatus used in this experiment was a large glass globe<sup>2</sup> about 20 inches in diameter, provided with two open necks at opposite ends of one diameter.

The upper neck was about two and a half inches wide and the lower about three-quarters of an inch wide.

The upper neck was plugged up with cotton wadding through which passed a glass tube which reached almost to the bottom of the globe inside. The end of the glass tube outside the globe was bent over at a right angle and passed through a rubber cork, which in turn fitted tightly into a wide glass tube filled with cotton-wool. By attaching an aspirator to this last tube, air could be sucked out of the globe from time to time, and fresh

<sup>1</sup> Marshall Ward : Recent Researches on the Parasitism of Fungi. Ann. Bot., 1905.

<sup>2</sup> The globe was of the same type as that shown in Fig. 2.

air drawn in through the filtering wad of cotton fixed in the neck of the globe.

The globe was supported over an ordinary earthenware pot, ten inches across at the top, by means of a wooden frame, which held it in such a position that, when the pot was filled with earth, the small lower neck of the globe was plunged one inch deep below the soil surface.

Eleven globes of the type just described were used in the first experiment.

Before starting the cultures, the globes were sterilized by formalin vapour in a closed chamber for 12 hours. On removing from the chamber, the two necks of each globe were plugged at once with sterile wads of cotton-wool, and the sterilized glass tube was inserted through the wadding in the large aperture.

The pots were then filled with good soil and the wooden frames placed on top of the pots in readiness to receive the globes.

Some clean sand was then sterilized and kept in sealed sterile tins.

When the above preparations were complete, the sterilization of the seed was carried out.

Two kinds of seed were used—some taken from single and some from double hollyhocks.

The plants from which the seed was taken were all diseased during the previous season.

The seeds were husked—i. e. removed from the carpels which enclosed them—placed in tap-water, and exhausted twice in succession by means of a Geryk pump.

They were then placed in 10 per cent. (commercial) formalin for five minutes, washed in three changes of sterile tap-water, and then transferred to sterile Petri dishes.

The seed was sown as follows:

A small heap of sterilized sand was first made on top of the earth in the centre of the pot. The seed was placed on top of this by means of a pair of sterilized forceps, and a little sterile sand was poured over the seed. The cotton plug at the bottom of one of the globes was removed and the globe pressed down into the sand so that the lower neck of the globe surrounded and enclosed the seed, which might now be looked upon as lying in a cylinder of sterile sand, surrounded by the glass neck of the globe, but with free access to the open space inside the globe for the growth of its shoot and a continuous layer of sand below it, through which the roots could find their way to the soil beneath. In this way the seed and the interior of the globe were protected from the entry of spores of all fungi, except those which could grow saprophytically in the soil and sand.

The seeds, and later the plants which grew from them, were watered with rain-water, which was poured on to the surface of the soil in the pots without disturbing the 'sand-lock'. Sufficient water reached the seed by

capillarity to ensure germination. On June 7, 1912, one seed was sown under each of the globes in the manner described above.

Six of these seeds failed to germinate or produced sickly seedlings, and were replaced by fresh sterilized seed about three weeks later.

Twelve seeds from the same source, but unsterilized, were sown in small pots as controls at the same time as the first sowings were made under the globes. Two of these seeds also failed to germinate, but were not replaced.

The globes were placed in line along a bench in an unheated greenhouse, and were sheltered from direct sun by means of blinds. The control pots were placed between the globes along the bench.

The remainder of the seed was sown in an open bed just outside the greenhouse.

By the end of August two of the plants in the globes had failed—due in one case to an accident.

The remainder of the 'globe plants' had from four to six leaves apiece, and varied from about the same size as the 'pot-controls' to twice that size.

The colour of the leaves was scarcely any lighter than that of the controls in pots, but the texture of the leaves was obviously thinner. None of the plants in globes or pots showed any rust.

The plants in the outside border, examined three days later, showed slightly fuller growth, and practically every plant had a few scattered rust pustules on one or more of its leaves.

One of these slightly diseased plants was potted up on September 3, in place of one of the 'globe plants' which had failed. The shoot and leaves of this plant were pushed through the small neck of the globe, which was lowered over it. The fate of this particular plant is interesting, as it shows that the conditions inside the globes were highly favourable to the development of the fungus. When first placed under the globe it bore three infected leaves; the largest of these showed 12 immature pustules, the second largest showed one mature pustule, and the smallest showed a couple of dozen undeveloped pustules.

By October 11 the three oldest leaves had died off, presumably killed by the fungus: of the remainder, two leaves, each about two inches in diameter, were covered with innumerable pustules, whilst three young leaves just emerging from the bud were, apparently, free from disease.

Three weeks later the entire plant was dead and covered with the remains of rust pustules.

This would seem to prove that the conditions inside the globes, though certainly abnormal, were not in any way such as would suppress the growth of any fungus latent in the tissues of plants growing inside the globes. Two features are also well worth noticing in the results just described: (1) that a certain development had to be reached by the young leaves before they could become visibly infected, and (2) that the density of pustules on the

expanded leaves was even greater than that shown in any of the photographs in Eriksson's paper of leaves suffering from a 'primary' outbreak of the disease.

To return to the main experiment; from the beginning of September onwards the controls in pots began to be much troubled by greenfly and later by red spider (*Tetranychus*), which much interfered with their growth, and necessitated treatment with paraffin wash. Notwithstanding this, two pustules were found on one leaf of one of these controls on October 11, and three weeks later another pot-control plant showed an equally light infection.

The control plants in the outside border continued to show more and more rust as the season went on, but even at the time when they finally died down, none of the plants showed that dense infection which Eriksson has ascribed to 'primary infection'.

The plants in globes were observed up till the beginning of December, when growth had ceased altogether, the greenhouse not being heated.

On December 4 each of these plants bore 8 to 9 leaves, of which the largest measured 3 inches across and was supported by a petiole 5 inches long.

No trace of rust had appeared on any of these plants, despite the proved suitability of their environment and the fact that, on Eriksson's expectation, the rust should have appeared, in bulk, by the end of September.

It was found, however, on this date that a few patches of mould were growing on the oldest leaves, which had dried up and fallen away from the crown.

These saprophytic fungi had doubtless made their way through the 'sand-lock' at the bottom of the globe.

From the middle of October to the beginning of December occasional Collembolae were seen in five of the globes. These live habitually in the soil and had doubtless worked their way up from below, and would be the means of introducing spores of saprophytic fungi.

During the whole of the experiment all the plants growing in globes had remained perfectly healthy, with the exception of one, which was recorded on September 3 as showing a small black-coloured patch on one leaf, and which subsequently developed two similar black-coloured patches on one petiole.

These affected leaves were removed through the top of the globe under sterile conditions, and were examined microscopically. No trace of rust could be found, and the black spots, which proved to be small areas of dead tissue, must be attributed to the action of Collembolae.

No further action was taken with the globes until the following spring, when, growth restarting, the aeration of the globes was recommenced.

At the end of April one leaf of one of the plants was inoculated with

spores taken from one of the 'pot-controls' growing near at hand. On the 20th of the same month several pustules of *Puccinia* were found on the underside of the inoculated leaf, and three other leaves on the same side of the plant showed a few pustules.

The experiment was finally brought to an end on May 22, 1913. On this date all the plants were removed from the globes and carefully examined. All had formed several new leaves during the spring, and the number of Collembolae was much increased.

None of the plants, however, with the exception of the artificially inoculated one just referred to, showed any sign of rust.

The 'pot-controls' were also examined on this date. These had kept their leaves during the winter and the rust had made steady progress. Three of these control plants are recorded as being 'heavily infected', two as being 'moderately infected', and the remaining four as 'slightly' or 'very slightly infected'.

The plants in the border outside had been grubbed up during the winter, and, consequently, no record is available.

#### *Summary of Results of Experiment 1.*

The cultures were started during June 1912, and were continued until the middle of May 1913. No rust appeared on any of the protected plants throughout the experiment, though, on Eriksson's expectation, a 'primary' outbreak might have been expected by the end of September at latest. The controls in the same house first showed rust on October 11, but only a few pustules. The rust progressed slowly throughout the winter, and by April 1913 the majority of these plants showed a fairly heavy infection. The controls in the bed outside first showed slight infection on September 3, and this increased as the season went on, but never reached 'primary' intensity.

One of the 'globe plants' was inoculated, towards the end of April, and gave a positive result.

At the beginning of September a slightly diseased plant, growing amongst the outdoor controls, was potted up underneath a globe. A month later some of the leaves of this plant showed extremely heavy infection, recalling Eriksson's description of 'primary infection'. After seven weeks the plant had been killed outright. The condition inside the globes would therefore appear to be favourable to the fungus.

#### *Experiment 2.*

In this series of cultures an attempt was made to improve on the conditions in the former experiments as follows: (1) by installing a system of continuous regulated aeration of the globes. (This would also have the effect of reducing the humidity of the atmosphere inside the globes.)



(2) by taking steps to exclude the entry of Collembolae, &c.

With these objects in view the globes were fitted with large cork bungs replacing the cotton wads in the upper apertures. Each cork was bored to receive three tubes. In the centre a short wide tube, ordinarily plugged with cotton-wool, but available for the passage of any instruments, &c., necessary for inoculating or for withdrawing single leaves for examination; on one side of the central tube a small inlet-tube, penetrating only a little way into the globe and with its exterior end passing into a wide tube, plugged with cotton, to act as a filter. The third tube was the one used for withdrawing the air: this one was bent to follow roughly the inside curve of the globe, and had its internal opening close to the bottom of the globe; the external portion of this tube passed into a cotton-plugged tube, designed to act as a germ-filter in case the aspirator should fail.

To the other end of this germ-filter was attached a tube which ran down into a small gas-jar and dipped below the surface of some water contained in the jar. A cork was inserted in the top of the gas-jar, and through this another short tube was connected by pressure tubing to a long gas-pipe, which passed in front of all the globes, and was, in turn, connected to an aspirator, which thus served to draw a continuous current of air through all the globes. The rate of the air current was equalized in each globe by watching the rate at which the air bubbled through the water in the gas-jars, and adjusting a pressure-clip, placed on one of the rubber connexions, so as to make the 'bubble rate' the same in each case.

Owing to the shape of the upper neck of the globes, it was found difficult to get a 'spore-tight' fit with the bung alone, and, to ensure this, it was found to be necessary to stretch a piece of rubber tissue tightly over the cork in each case before pushing it in.

The general appearance of these globes, when in action, is shown in Fig. 2.

In order, if possible, to exclude Collembolae, the earth used in this experiment was sterilized before starting, and, in addition, a layer of sterilized sand, one inch deep, was spread over the entire surface of the soil immediately before planting the seed.

Finally a piece of fine muslin was tied over the bottom of the pot to protect the drainage hole.

The soil sterilization was carried out as follows:

The soil was thrown into a large metal bin and sprinkled during the process with toluene; cloths were then put on top and weighted down, and the bin was covered and left for 20 hours. At the end of that time the soil was tipped out on a clean cement floor, and stirred over four times in the two succeeding days.

It was then put straight into the pots, which, together with the crocks, had been sterilized previously in a strong solution of formalin.

In spite of these precautions, Collembolae did again appear, though very sparingly. This must be attributed to the fact that the plants were watered with tap-water owing to the impossibility of sterilizing sufficient water to meet the daily needs of the plants in summer.

The globes and tubes were sterilized with formalin vapour as before, and the corks and all rubber parts were immersed in rectified spirit for

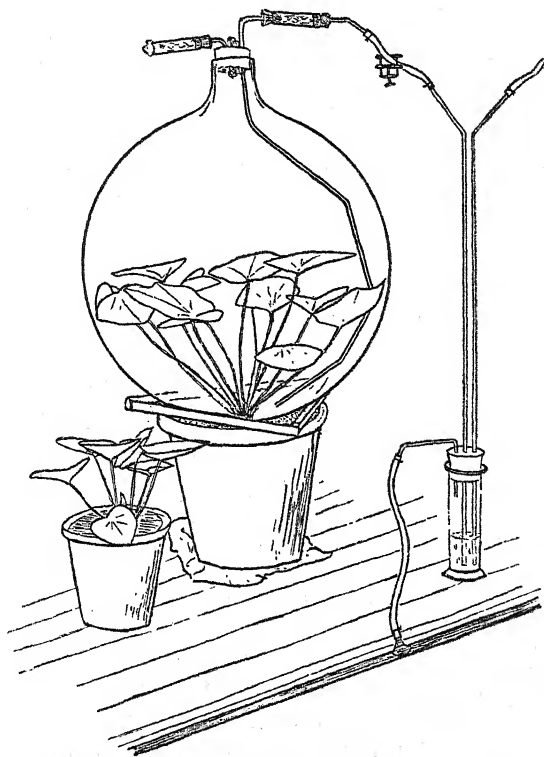


FIG. 2. Hollyhock Globe No. 9 and Control Pot No. 7 (from a photograph taken on June 27, 1914).

the same purpose. In addition to the formalin treatment, all the glass-ware was rinsed over with rectified spirit shortly before starting the experiment.

The seed used in the globes and pot-controls for this experiment was collected in October 1913 from some double hollyhocks with reddish magenta flowers, which had shown a considerable amount of rust on leaves, stems, and calyx, and also on the carpels themselves during the foregoing season.

In order to avoid trouble from seeds failing to germinate, they were

not placed under the globes until the radicle had begun to push through the seed-coat. Otherwise the method of sowing the seed was the same as in the first case.

Sterilization was effected in 1 per cent. mercuric chloride for 8 minutes, and the seeds were subsequently washed in six changes of sterile tap-water before being left to germinate under sterile conditions.

The seed was sown under the globes on November 3, 1913, and the house containing the plants was kept at a warm equable temperature throughout the winter. Ten globes were employed. Ten 'pot-controls' were also sown on the same date, sterilized soil and seed of the same kind being used. Unfortunately the germination of the seed was not tested in this case, with the result that seven of the pots had to be resown with germinated seed three weeks later. The seed and method of sterilization in this last case were the same as before.

In addition to these 'pot-controls', a large quantity of hollyhock seed was sown in boxes to act as further controls.

On October 17 a boxful of seed, collected by Mr. H. Festing Jones from hollyhocks growing on Monte St. Julian in Sicily, was sown, and the resulting plants will be referred to in the following pages as *Sicilian hollyhocks*.

On November 3 three large seed boxes of each of the following were sown:

'*Althaea rosea*, fl. pl.'

'*Althaea rosea*, fl. pl. (Chater's Superb).'

The former will be referred to as *Double German*, and the latter as *Chater's* in this account.

It should be noted that in no case, in these 'box-controls', had the seed-producing parents been seen. The Sicilian seed was sown owing to the reputed immunity of these plants from the attacks of rust. The seed of the two pink varieties was imported from Germany and selected on Prof. Eriksson's advice as being most likely to show the phenomena which he had described in his paper.

The seed in boxes in all cases germinated well.

Early in February 1914 and again in March the house was fumigated with nicotine without injurious effect to the foliage of any of the plants. On February 7 the benches, &c., were scrubbed down with formalin and water; this latter operation caused the controls to exhibit slight 'fumigation injury'.

Throughout the year the cotton plugs and rubber connexions frequently required changing.

All plants grew on steadily during the winter and subsequent summer, and by the end of April 1914 the plants growing in boxes were cramped for lack of room. Twelve Sicilian plants were therefore potted up, as were

also 18 plants of each of the two double pink varieties. These pots were kept in the same greenhouse as the globes.

Two dozen plants of each of these three kinds were planted out at the same time in a garden half a mile distant.

On May 1, 1914, each of the plants in the globes had from 7 to 10 leaves, the largest of which measured  $4\frac{1}{2}$  inches across and was borne on a stalk 10 inches long. The 'pot-controls' were somewhat smaller and bore only about six leaves apiece, and the plants which had been started in boxes were smaller still, owing to a period of overcrowding.

At this date the plants in the globes were lighter in colour and their leaves were less rugose than those grown in the normal way, but they appeared to be more vigorous than the controls.

At the end of June the difference in size between the 'globe' plants and the 'pot-controls' was even more striking, as will be seen in Fig. 2, which is drawn to scale from a photograph taken on that date.

At this date no rust had appeared on any of the plants, including those which had been planted out.

In the early part of July, during a sudden burst of sunshine on an otherwise cloudy day, most of the fully expanded leaves inside the globes were scorched by the sun. The worst affected of these subsequently died, and, dropping to the bottom of the globes, became infested with saprophytic moulds.

These dead leaves were removed in order to keep down the saprophytic fungi, and in the course of this operation the globes had to be opened for a short time.

At the end of August the growth of the plants in globes was still good compared with that of the four sorts of controls in pots in the same house, which had suffered a good deal from the attacks of red spider.

All the plants inside the greenhouse still remained free from rust, despite the fact that the plants which had been set out in the open now showed a plentiful infection. These latter had all sent up flowering spikes, and the rust was distributed over all except the very youngest leaves, which were unaffected except in very rare instances.

The pustules on the older leaves at the bottom of these plants were in a more advanced stage than those farther up, and were obviously the result of an earlier infection.

The plants which had been started in boxes and subsequently potted up had made very poor progress, and on August 22 they were repotted and stood outside under a north wall.

On September 5, ten months after the start of the experiment, the plants in the greenhouse still showed no sign of disease, though this was three months after the latest possible expectation on the *Mycoplasma* Hypothesis.

To test whether the conditions were entirely inimical to the growth of the fungus, a bunch of heavily infected hollyhock leaves was shaken in all parts of the greenhouse. Seventeen days later, as the result of this, six of the pot-controls showed one pustule each. In five cases out of six this pustule appeared on a fully-developed leaf; in the remaining one case it occurred on a young leaf. The plants in the globes remained unaffected.

The plants in pots, standing in the open, were also examined on this date (Sept. 22) and were found to show the beginnings of infection; 60 per cent. showed a few pustules, almost entirely on the middle-aged, fully expanded leaves.

The experiment having now run so long (10½ months), it was found that the rubber-tissue between the cork and the neck of the globes had perished, and the apparatus could no longer be relied on effectively to exclude air-borne spores.

It was decided, however, not to replace the perished rubber, but to keep the apparatus running whilst certain inoculation experiments were tried.

No observations were made on the plants in the globes during the next month, with the exception of one which was inoculated on September 29 and again on October 6 by spraying it with a suspension of germinated rust-spores.

These spores had been germinated at the bottom of a dish of sterile tap-water and were examined before they were sprayed on to the plant. In all cases, as might be expected, they had germinated in the form of 'oidia'. The results of both these inoculations were negative.

On October 28 the plants in the globes were again carefully examined, and it was found that in two cases plants were infected with rust. Neither of these two plants had been inoculated, and the inference is that they had been infected by air-borne spores owing to the faults which had developed in the apparatus as described above.

In both these cases the globes were removed, and the plants cut off at the crown and carefully examined.

The results of this examination are important, as they confirm the opinion that the infection was an external one, and not a manifestation of 'primary infection'. The fact that the pustules were of various ages shows that the disease had been running its course for some time before the plants were examined.

In the following table, 'G.G. 2' and 'G.G. 3' are the reference numbers of these cultures, and the leaves are in each case numbered from 1 upwards, starting with the oldest.

TABLE II.

Ref. No.	No. of leaf.	Dimensions (in inches).		Extent of disease.	Remarks.
		Length of lamina.	Length of petiole.		
G.G. 2	1	3½	9	No rust.	
	2	"	"	"	
	3	"	"	One very small young pustule.	
	4	"	"	One very small young pustule.	
	5	"	7	Two young and one fairly old pustule.	
	6	"	7	One fairly old pustule.	
	7	3	7	Five young and one old pustule.	
	8	"	"	A fair number of pustules scattered irregularly over the leaf, young and old mixed indiscriminately.	
	9	"	"	Fair number, mostly young, but one old one near edge of leaf.	
	10	"	"	Fair number. Equal number of old and young pustules.	
	11	"	"	Fewer than above. All old except two.	
	12	"	"	About 150. None more than moderately old; majority are young.	} These two leaves stood up in the centre of the globe.
	13	"	"	About 150. Mostly fairly old.	
	14	2½	5	Five young pustules. One fairly old.	
	15	1	1	} No pustules.	
	16	"	"		
G.G. 3	17	"	"	"	
	1	4	10	No rust.	
	2	"	"	"	
	3	"	"	"	
	4	"	6	Twelve young pustules scattered over surface.	This leaf was growing close to No. 8.
	5	"	8	No rust.	
	6	"	"	"	
	7	"	"	"	
	8	"	6	An elongated area about 2 inches long and 1 inch broad, situated in the extreme proximal portion of the leaf, was densely crowded with pustules varying in age from moderately old to those which were only just breaking the surface. The rest of the leaf bore about 20 pustules only, scattered irregularly except for some 5 or 6 grouped in a rough circle. These last were all old pustules.	The biggest pustule from the circular colony of old pustules was examined under microscope. Found that the great majority of teleutospores had already germinated and dropped their spores.
	9	4½	"	One young pustule only.	
	10	"	"	Two pustules. One old and one young.	
	11	"	"	About 9 pustules of varying age.	

TABLE II (*continued*)

Ref. No.	No. of leaf.	Dimensions (in inches).		Extent of disease.	Remarks.
		Length of lamina.	Length of petiole.		
G.G. 3	12	4½	6	About 9 pustules of varying age.	
	13	"	"	About 9 pustules of varying age.	
	14	2½	4	No rust.	
	Expanding crown leaves.	—	—	"	

Additional evidence of these being cases of outside infection is afforded in the further behaviour of 'G.G. 3'. The globe was replaced over the stump of this plant, from which all the leaves had been removed for examination. In due course new leaves developed from this stump, and these remained free from rust until six months later, when the globes were finally removed.<sup>1</sup>

It has been mentioned above that one of the plants in globes—No. G.G. 8 in the series—was inoculated with a suspension of oidial spores during September and October, but without result. Later on, further attempts at artificial inoculation were made on some of the other plants, but in all cases the result appeared to be negative.

A summary of the history of all the plants in globes from October 26 onwards is given in the following table. It will be observed that all the plants which had been infected finally developed rust, but only after an interval that made it appear improbable that the outbreaks were directly attributable to the inoculation.

The fact that so many plants—six out of ten—did develop disease before the end of the experiment may seem remarkable, but, as has been stated above, the apparatus was no longer reliable, and it should also be borne in mind that a continuous current of air was being sucked into the globes through whatever channel offered the least resistance, and that the control plants on the bench alongside bore rust pustules throughout this period.

With the exception of G.G. 2 and G.G. 3, all the globes had been last examined about a fortnight before the date on which they first showed rust in each case and had been described as 'free'.

<sup>1</sup> In this connexion it is interesting to note that Massee, in 'The Diseases of Cultivated Plants', states that plants which have once recovered from the disease are immune from further attacks.

As the result of two series of experiments on this point I have satisfied myself that no such acquired immunity exists.



TABLE III.

Ref. No.	Inoculations.		First appearance of rust.	Remarks.
	Date.	Method.		
G.G. 1.	26.10.14	Infected leaves shaken over open neck of globe.	8.12.14. Four middle-aged leaves show few pustules (average, 9 per leaf).	Rust increased rapidly, and by 12.1.15 all leaves, except the very smallest, were heavily rusted.
G.G. 2. } G.G. 3. }	—	nil	Details given above in Table II.	
G.G. 4.	19.10.14	Sprayed with suspension of teleutospores which had germinated to produce both sporidia and oidia.	8.12.14. Eleven leaves show pustules, only the unexpanded leaves being free. Maximum number of pustules on one leaf, 64. Average number 10 per infected leaf.	Rust increased rapidly, and by 12.1.15 the plant was mostly dead.
G.G. 5. } G.G. 6. } G.G. 7. }	—	nil.	nil.	Last examined 13.5.15, when they were still free from disease.
G.G. 8.	29.9.14	Sprayed with suspension of teleutospores which had germinated to form oidia.	20.11.14. Five youngish leaves affected. Maximum number of pustules on one leaf, 70. Average number, 30 per infected leaf.	Rust increased rapidly till by 12.1.15 all leaves were killed with the exception of those still unexpanded.
	6.10.14	Ditto.		
G.G. 9.	—	nil.	nil.	
G.G. 10	—	nil.	24.12.14. Three leaves (one fairly old and the other two middle-aged) show one or two incipient pustules.	Progress of disease rapid. By 12.1.15 most of the leaves bore numerous pustules, and by 5.3.15 the plant was dead.

It will be noticed that in none of these cases is the initial severity of the outbreak sufficient to place the plants in Eriksson's category of 'primary infection'. They appear, rather, to be the result of separate infections in each case, and in the case of plants which had been artificially inoculated may have arisen from teleutospores that had germinated some days after their introduction into the globes.

A final word is necessary as to the behaviour of the controls during the last stages of the experiment.

The fate of those which were planted out in April 1914 has already been described.

Those of the three kinds (Sicilian, Chater's, and Double German) which had been grown in pots under a north wall in the open first showed a few pustules on September 22. In this case also the disease continued to increase, but more slowly, until the plants died down for the winter.

Finally, as regards the pot-controls which were kept throughout in the greenhouse: these, as shown above, failed to show any rust until artificially inoculated. They were much attacked by various pests, and their growth much stunted in consequence. The amount of rust present on them increased slowly up till December 18, 1914, but never exceeded more than a few pustules per plant. After that date the amount of infection decreased, until in March 1915 only one plant remained infected.

*Summary of Results of Experiment 2.*

The experimental plants were kept under observation for a protracted period, far beyond that claimed by Eriksson as necessary for the development of 'primary infection'.

Eriksson's period is three months from the time of sowing, when growth is continuous as in this case. In this experiment the first appearance of rust was 10½ months after sowing.

The observed deterioration of rubber connexions eventually admitted of outside infection.

In all, six plants out of ten in the globes developed disease. In three of these instances attempts—apparently unsuccessful—at inoculation had been made, and it is possible that the eventual infection was due to this rather than to contamination from outside. In all six the infection, to begin with, presented a 'secondary' rather than a 'primary' *facies*. It soon developed an intensity equal to that of 'primary infection'. One of the six plants cut down to the root and allowed to sprout again showed no recrudescence although watched for six months.

The results indicate strongly that the protected plants did not suffer from 'primary infection', though raised from seed in which, according to Eriksson's views, 'primary infection' would have been anticipated.

The control plants also in no case gave the phenomena of 'primary infection', though some, at least, were raised from seed taken from plants which were known to have been heavily infected.

Certain of the controls, when once the disease had made a start, developed the rust with a prodigality equal to that of 'primary infection'. Others remained only slightly infected to the end. The comparative immunity of these latter plants may be attributed with little hesitation to the stunting effects of red spider.

The behaviour of these particular controls contrasts strongly with that of the plants in globes. Both series of plants were slow to show infection at all, but whereas the controls, stunted by insect attacks, showed little tendency to a further development of the fungus, the plants in globes succumbed with a rapidity greater than any noted under the most favourable conditions outside.

# APPENDIX.

## GERMINATION EXPERIMENTS WITH SPORES OF *PUCCINIA MALVACEARUM*.

### Series I. (July 1914.)

Spores scraped from young pustules on to surface of water in watch-glasses.

Some of the spores sank ; others remained floating on the surface.

Only such pustules used as were brown in colour or showed only a faint trace of grey.

Pustules taken from six different hollyhock plants, all of which were growing in the garden, and had been naturally infected.

Nine cultures made :

Culture.	Spores on surface of water.		Spores at bottom of water.	
	Germination.	Type.	Germination.	Type.
1. Sterile tap-water	Good.	Mostly oidial ; some sporidial.	Good.	Entirely oidial.
2. Distilled water.	Poor.	Mostly oidial ; one sporidial.	Poor.	" "
3. " "	Fair.	Mostly oidial ; some sporidial.	Moderate.	" "
4. Sterile tap-water.	Fair.	About equal proportions. One spore showed different germination from its two cells.	Fair.	" "
5. " "	Moderate.	Mostly oidial.	Good.	Entirely oidial.
6. " "	Poor.	Mostly oidial ; some sporidial.	—	—
7. " "	Good.	Mostly sporidial ; some oidial.	Good.	Entirely oidial.
8. " "	Very poor.	Apparently all oidial.	Good.	" "
9. " "	Poor.	" " "	Poor.	" "

### Series II. (July–August 1914.)

Entire pustules removed from leaves of naturally infected hollyhock plants, and placed on or below the surface of water in watch-glasses. Pustules taken from 3 different plants, growing in open bed.

Culture.	Spores in pustules on surface.		Spores in submerged pustules.	
	Germination.	Type.	Germination.	Type.
1. Sterile tap-water.	Good.	1-4. Mixture of sporidial and oidial ; the former preponderating.	Good.	All oidial.
2. " "	"		"	"
3. " "	"		"	"
4. " "	"		"	"
5. " "	"	5-7. Mixture of sporidial and oidial. In two or three cases spores lying just on the surface of the water showed different types of germination from the two cells of the spore (see Fig. 1).	"	"
6. " "	"		"	"
7. " "	"		"	"

Culture.	Spores in pustules on surface.		Spores in submerged pustules.	
	Germination.	Type.	Germination.	Type.
8. Sterile tap-water.	Good.	Portion of pustule above water-level produced a great quantity of sporidia. Where in contact with the water at the edges it was mostly oidial.	Good.	All oidial.

*Series III.* (May 1919.)

Young pustules teased out under surface of distilled water in watch-glasses.

Pustules from leaf of wild plant of *Malva sylvestris* showing rather heavy natural infection.

Three cultures made.

*Result.* Germination in all three cases was poor, and two cultures never got beyond the production of very short undifferentiated germ tubes. In the remaining culture long thin tubes were produced, which in one or two cases divided up to form oidia.

*Series IV.* (May-June 1919.)

Two diseased leaves of a naturally infected wild plant of *Malva sylvestris* removed and supported in such a way that one half of each leaf was immersed in water whilst the other half remained exposed.

The whole apparatus was covered with a bell-jar to ensure a moist atmosphere around the exposed portions of the leaves.

The older-looking pustules were removed before starting the experiment.

## RESULTS.

*Leaf A.*

Moderately heavy infection.	<i>Submerged portion.</i>	Germination moderate and entirely oidial.
	10 pustules examined.	
	<i>Exposed portion.</i>	Germination excellent in 5 cases and moderate in remaining 7. 99 % typical sporidial. No typical oidia.
	12 pustules examined.	

*Leaf B.*

Very heavy infection, which had developed gradually.	<i>Submerged portion.</i>	4 had germinated very well and produced oidia only, except for a very few tubes resembling abortive promycelia. 7 germinated poorly and produced only abortive tubes suggesting promycelia which had failed to develop. 1 failed to germinate. 1 germinated well and produced sporidia in quantity.
	13 pustules examined.	
	<i>Exposed portion.</i>	3 germinated poorly and produced sporidia only. 9 germinated well, producing sporidia only.
	12 pustules examined.	

Series V. (May-June 1919.)

Two leaves from a plant of *Malva sylvestris* growing next to the one employed in Series IV. Leaves treated as in Series IV.

RESULTS.

Leaf C.

*Submerged portion.*

Moderately heavy infection.

12 pustules examined.

- 6 germinated well, but for most part produced barren tubes intermediate between the two forms. A few oidia were produced, but no sporidia.
- 2 germinated rather poorly, producing a fair number of oidia, the rest being as above.
- 3 germinated poorly, producing mostly barren tubes as above, and also a few sporidia.
- 1 germinated fairly well, producing mostly sporidia.

*Exposed portion.*

12 pustules examined.

- 4 germinated well; the rest badly. Sporidia only were found in every case.

Leaf D.

*Submerged portion.*

Lightly infected leaf.

10 pustules examined.

- 2 germinated well, forming oidia only.
- 1 germinated well; mostly oidia, but a few sporidia also.
- 1 germinated moderately well, giving a few oidia and sporidia and a lot of barren abortive tubes.
- 4 germinated poorly, producing mostly abortive tubes, and occasionally oidia or sporidia.
- 2 failed to germinate.

*Exposed portion.*

10 pustules examined.

- 8 germinated well and 2 poorly. Exclusively sporidial.

Pairs or series of leaves taken from single plants. Some of these leaves were totally submerged; others were exposed to moist air.

The others entirely exposed to moist air.

Leaf.	Description.	Treatment.	Results.
E.	Five yellow cushions representing pustules not yet broken through. From a hollyhock plant growing in outside border and showing slight infection.	Submerged.	Leaf began to decompose before pustules matured.
E. 1.	About a dozen very young pustules. From the same plant as leaf E.	Exposed.	7 pustules examined. Germination good. Sporidia only.
F.	About 50 pustules, chiefly collected in one corner. Of these, 7 had just broken through and were marked with Indian ink. From naturally infected plant of <i>Malva sylvestris</i> growing in the open.	Submerged.	Leaf began to decompose before marked pustules germinated. 5 of the other pustules examined. Germination poor, but oidal only.

200 *Bailey.—Puccinia malvacearum and the Mycoplasma Theory.*

<i>Leaf.</i>	<i>Description.</i>	<i>Treatment.</i>	<i>Results.</i>
F. 1.	20 very young pustules. From same plant as F.	Exposed.	11 pustules examined. Germination mostly good and sporidial only. In two cases germination was very poor, and the promycelia were malformed, but these also produced sporidia.
G.	About 40 young pustules evenly distributed over leaf-surface. From plant of <i>Malva sylvestris</i> , showing fairly heavy natural infection. Plant growing in the open.	Submerged.	The leaf showed fairly advanced decomposition before much germination of spores had taken place. Those which germinated produced oidia.
G. 1.	Similar leaf from same plant.	Exposed.	18 pustules examined. Germination good in all cases. Sporidia only produced.
G. 2.	Two yellow cushions not yet broken through the leaf-surface. From same plant as leaf G.	Submerged.	Leaf decayed before pustules matured.
G. 3.	About 20 very young pustules. From same plant as G.	"	Leaf decayed before majority of pustules came to maturity. 5 pustules examined. Of these one showed good germination and two fair germination. Oidia only produced. The remaining two germinated badly. One of these last produced oidia only; the other a few sporidia as well.
G. 4.	About 20 young pustules. Leaf from same plant as G.	Exposed.	6 pustules examined. All gave good germination, except one which germinated very badly. Sporidia produced in every case.
G. 5.	Similar leaf to last. From same plant.	"	5 pustules examined. Germination good. Sporidia only produced.
G. 6.	20-30 pustules irregularly scattered over surface. From same plant.	Submerged.	9 pustules examined. All, except one, germinated well. 5 gave oidia only. 2 gave a few sporidia in addition to the oidia. 2 produced a large number of oidia, a few sporidia, and a fair number of germ tubes suggesting abortive promycelia.
G. 7.	Similar leaf to last. From same plant.	"	5 pustules examined. Germination good in each case. Oidia only occurred, except in one case where a few sporidia also appeared.
G. 8.	Similar leaf to last. From same plant.	"	3 pustules examined. Oidia only produced.

# A Contribution to our Knowledge of the Vascular System of the Genus *Equisetum*.

BY

KATE BARRATT, M.Sc.

With Plates VI and VII and twenty-four Figures in the Text.

IN spite of the large amount of work already published on the subject of the anatomy of *Equisetum*, of which an excellent chronological account has been given by Lady Isabel Browne (1), there still seems to be a considerable lack of agreement as to the nature and origin of the various parts of the vascular system. The presence or absence of centripetal wood is one of the most prominent of these questions. As a contribution towards the solution of some of these problems it was decided to undertake a more thorough investigation of the anatomy of the sporeling, and to bring together and amplify our information on the development of the vascular system.

Hofmeister (3), as long ago as 1852, included a brief reference to this subject in his account of the genus. He gave an excellent figure of a young sporeling at the stage when its first lateral shoot was developing, but he does not refer in detail to the nature of the vascular structure.

In 1899 Jeffrey (4) described the development of sporelings of *Equisetum hiemale* and *E. limosum*, but only dealt generally with the anatomy of the young plant.

It was hoped that a further investigation of the sporeling anatomy would throw light upon the following points:

1. The nature of the stelar anatomy of the primary axis.
2. The phylogeny of the internodal bundle, with special reference to the metaxylem.
3. The elucidation of the vascular system of the node, in relation to the internode and the question of secondary thickening.

## METHODS.

One of the reasons for the incompleteness of our knowledge of these plants in their young stages probably lies in the fact that the sporelings are very slender and fragile, and that previous investigations have been largely confined to the examination of microtome sections.

Although serial sections have been used in this investigation, another



method, described below, has been employed to determine the relations existing between integral parts of the vascular system.

The species which has been most carefully worked through is *E. arvense*, owing to the ease with which the spores of this species could be obtained and the young plants raised under cultivation. But sporelings of *E. maximum* and *E. limosum* have also been employed.

The spores were sown on light soil immediately after gathering and the pots were kept in a frame at the ordinary temperature out of doors.

The prothallia appeared in the course of two or three days after sowing and the first young sporophytes were visible two months later. The prothallia were carefully removed from the soil, washed and pickled in 75 per cent. spirit. An abundance of material was thus available.

Plants of different sizes, varying from those still buried within the prothallial lappets to those showing three or four shoots, were detached from their prothallia and treated for twenty-four hours with a solution of eau de Javelle in the cold. At the end of that time the whole sporelings were transparent and extremely fragile. They were then washed in water and stained with ammoniacal fuchsin.<sup>1</sup>

The material was allowed to remain in the staining fluid for 24 hours or longer; it was then washed with alcohol several times and during this process the red colour appeared in the lignified tissues. The specimens were then dehydrated, cleared with oil of cloves, and mounted in Canada balsam. For some purposes it was found convenient to mount the stained specimens in glycerine jelly or euparal, the lower refractive index rendering the cellulose walls of the parenchymatous cells more visible.

Thin slides were employed for mounting, so that when desired the specimens could be examined from either side.

The result of this treatment is to render the whole sporeling transparent, and the lignified tissue, being vividly stained, stands out as a complete internal skeleton; it was possible to follow the course not only of the vascular strands but of their individual components, and thus to correct or confirm conclusions drawn from the examination of serial sections. Since the fuchsin also stains cuticularized membranes, the characteristic bands on the radial walls of the endodermal cells appear as a connected network (Plate VII, Fig. 1).

The same method of clearing and staining was applied to the apices and mature shoots of adult plants with equal success. Before clearing the material each apex or shoot was split longitudinally into two halves and so mounted that the interior of the stem was uppermost. These thick specimens were mounted in Canada balsam in cells constructed for the purpose.

<sup>1</sup> This stain is prepared for use by adding 5 per cent. solution of basic fuchsin in alcohol to strong ammonia 0.880, so long as the liquid remains colourless; refer Zimmerman, Botanical Microtechnique, § 271.

EXTERNAL MORPHOLOGY OF THE YOUNG PLANT OF  
*EQUISETUM ARVENSE*.

As already stated, the young plant of *Equisetum* appears above the level of the prothallus about eight weeks after the sowing of the spores.

The young sporophyte consists of a slender primary stem continued below as a thin elongated root. At the level of the prothallus the first node is found bearing two or three leaves, their bases fused into a sheath and their distal parts free. Just below the level of the first node, one can make out the embryonical organ, the foot. It appears as a protuberance on one side of the axis. The primary stem continues to elongate until several nodes are formed, each with two or three leaves, the larger number being the more common. The number of nodes formed by the first shoot is very variable, depending largely upon the general strength and external conditions of the young plant (Pl. VI, Fig. 1, *a-g*).

At a very early stage in the latter's development, a bud is formed which gives rise to the first branch. It appears below the first node on the side remote from the foot, pushing its way out below the leaf-sheath. Its position on the axis varies considerably, as may be seen by reference to Pl. VI, Fig. 2, *a* and *b*; sometimes it is situated at a considerable distance below the attachment of the leaves and sometimes almost at the node. This bud, on emerging from the primary axis, takes a sharp turn downwards before ultimately taking the upward course of an aerial stem. Before the young branch makes its way through the cortex of the primary axis, it gives rise to an adventitious root which immediately grows out into the soil.

Other lateral buds may be developed sparingly at the upper nodes of the primary stem, but these develop much later if at all.

The secondary axis in its turn gives rise to a lateral bud with its associated root, and this process may be repeated until normally three or four upright shoots have appeared. There is a tendency for the later-formed shoots to be longer and more robust, with a gradually increasing number of leaves at the nodes (Pl. VI, Fig. 1, *a-g*). There is, however, no great regularity in this last respect, the second shoot showing generally three leaves, and the third and fourth exhibiting four leaves in a whorl.

According to Jeffrey (4) as many as 12 erect shoots are produced in *E. hiemale* before the first rhizome makes its appearance, but in these cultures of *E. arvense* the number of aerial shoots has never exceeded five and the majority show three. The fate of the later-formed branches is dependent upon the conditions under which the sporelings are reared. In the plants resulting from thinly-sown spores, three aerial shoots were generally formed, and the fourth and fifth were destined to be rhizomes. The latter elongate very quickly and grow horizontally through the soil,

giving off aerial branches at nodes some distance away from the primary axis.

In the later-sown cultures there was a tendency for an earlier preparation for winter conditions. This is usually brought about by the formation of one or two tubers in the place of rhizomes (Pl. VI, Fig. 1, *e, f*).

In these artificial cultures it is well to bear in mind that the degree of development of the young plants is largely dependent on the external conditions, and especially on the density of the culture. Plants closely crowded are correspondingly hampered and restricted in development.

All the young plants figured in Pl. VI, Fig. 1, *a-g*, were taken from the same culture and were from prothallia of the same age.

It is clear that the result of this early development of branches is the formation of a sympodium which is constructed from the bases of the first-formed aerial shoots. A distinct subterranean region is thus formed compounded from the bases of the second, third, and fourth aerial shoots. We shall see that the vascular structure of this region differs very strikingly both from the axis of the aerial stem and from that of the ordinary rhizomes.

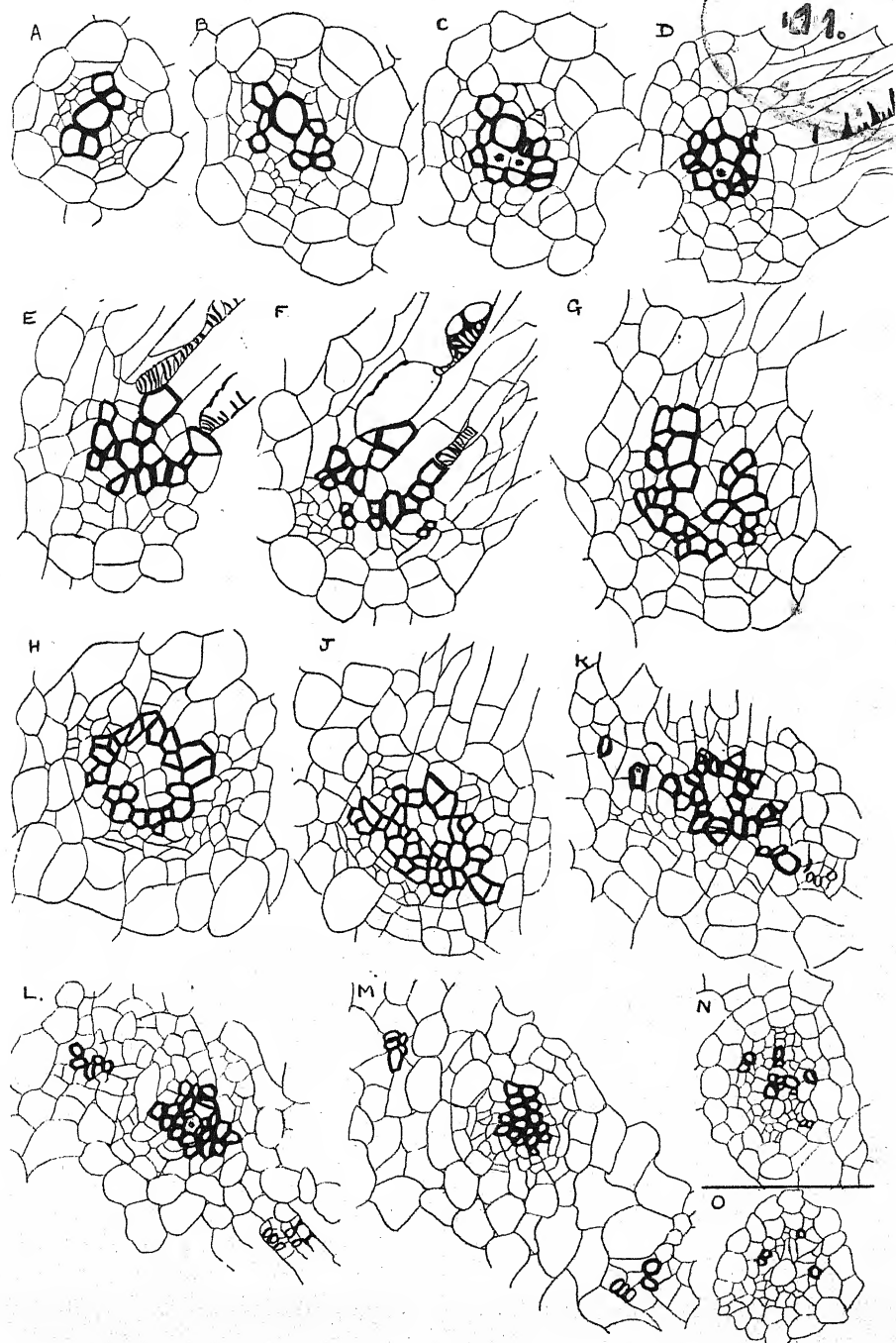
At the close of the first season's growth the young plants may show quite extensive branch systems. The primary and secondary axes generally die down early, and always before the end of the first season's growth.

#### ANATOMY OF THE SPORELING.

The primary root exhibits a simple diarch structure, each xylem strand being composed at first of a single series of tracheides. As the root approaches the region of the foot the xylem is increased by the addition of relatively wider but shorter tracheides with coarsely reticulate markings, strikingly different from the typical long spiral and annular elements of the root. The normal elongated elements thus give place to a more or less solid mass of short strongly thickened tracheides which are of the type so constantly met with in the nodal regions of the mature plant.

Text-fig. 1 illustrates a series of transverse sections taken through a sporeling which has already produced a second aerial axis. From these it will be seen that the stele enlarges as it passes from the root upwards, and that the increase of tissue is due to the addition to the xylem of both parenchymatous cells and tracheides. One or two parenchymatous elements are found towards the centre of the mass (Text-fig. 1, *C*), but at this stage they are neither numerous enough nor sufficiently constant in position to constitute a pith.

Under a high magnification these tracheides often exhibit an unusual appearance when seen in transverse section; the walls bordering on parenchymatous cells are thin and un lignified, a condition which tends to over-



TEXT-FIG. 1 (A-O). Series of transverse sections through the base of the primary axis of a sporangium—passing from the root upwards to the base of the first internode.  $\times 275$ .

emphasize the proportion of parenchyma present in the xylem (Pl. VI, Fig. 5). The explanation may be found by reference to similar elements in the cleared preparations. Here they are seen to be short wide tracheides tapering sharply at both ends, with an irregular open reticulum of thickening, very large pits being found on the walls bordering parenchymatous cells; hence when a transverse section traverses one of these pits the wall appears quite unthickened (Pl. VI, Fig. 6).

At this level the first indication of the presence of the branch supply makes its appearance (Text-fig. 1, E and F). A gap is noted on one side of the xylem mass and the parenchymatous elements towards the centre of the stele come into direct communication with the central well-developed pith of the lateral shoot. The xylem of the latter, where it joins on to the main axis, is arranged in an unbroken cylinder (Text-fig. 1). As the series of sections is traced upwards the gap in the xylem cylinder is closed by the appearance of elements belonging to the upper part of the branch stele, thus leaving no lateral gap in the wood above the branch trace. Gradually the primary vascular axis contracts in width and the proportion of parenchyma in the pith is reduced until at the level of the leaf-traces it has returned to the condition obtaining prior to the origin of the branch, and ultimately a solid protostele is found (Text-fig. 1, G-M).

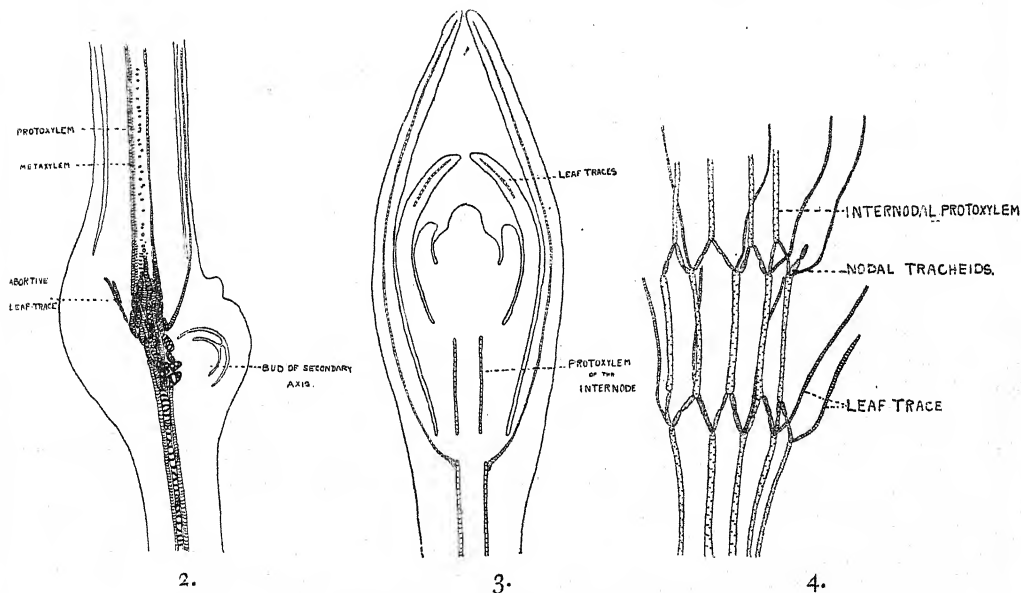
The attachment of the leaf-traces is not accompanied by any disturbance in the arrangement of the vascular tissue. The number of leaf-traces present at this first node is variable, not always agreeing with that of subsequent nodes on the same shoot. There seems to be a general tendency for one at least of the three leaf-traces typically present to be either reduced to a very short strand or to be absent altogether. This reduced trace is usually that adjoining the foot, but sometimes it is the one on the side towards the first lateral shoot (Text-fig. 2). There is a very decided tendency for the three leaf-traces to join the stele at different levels and thus to have a spiral arrangement (Pl. VI, Fig. 4). This might suggest that the very pronounced whorled character of the phyllotaxis of the adult plant may not be in itself a primitive character although it has been long established.

The transition from the solid protostelic condition, just described, to that of the internode above is sudden, and is marked by the change from short wide reticulate tracheides to a few much elongated annular ones (Text-fig. 1, M, N, and O).

As Vidal (6) and others have pointed out, the stem may be regarded as built up of a series of segments, each consisting of a node with an accompanying internode. This conception is certainly supported by the manner in which the vascular tissue develops. The internodal protoxylem strands are the first to differentiate, as Quéva (7) has shown from his study of transverse sections. The strands correspond in number with the leaves

of the node above and are situated on the same orthostiches. Examination of cleared preparations shows that the first element to differentiate in each strand is a tracheide which often extends through the whole length of the internode, or there may be two tracheides joined end to end. Further differentiation results in the addition of other tracheides in a more peripheral position.

The traces in the leaves develop concurrently, the tracheides first differentiating in the distal region, but very soon linking up with the protoxylem of the internode, either directly or by the intervention of one or two short coupling tracheides (Text-fig. 3, and Plate VII, Fig. 3). The alter-



TEXT-FIG. 2. Longitudinal section of basal region of primary axis, showing an early stage in the development of the first lateral bud. Note abortive leaf-trace adjoining foot.

TEXT-FIG. 3. Diagram illustrating the apex of a sporophyll, showing the independent development of the internodal xylem strands of successive internodes. The nodal tracheides have not yet differentiated.

TEXT-FIG. 4. Diagram of apex of older shoot illustrating the xylem development prior to the differentiation of the metaxylem. The nodal tracheides already link up the successive internodal strands.

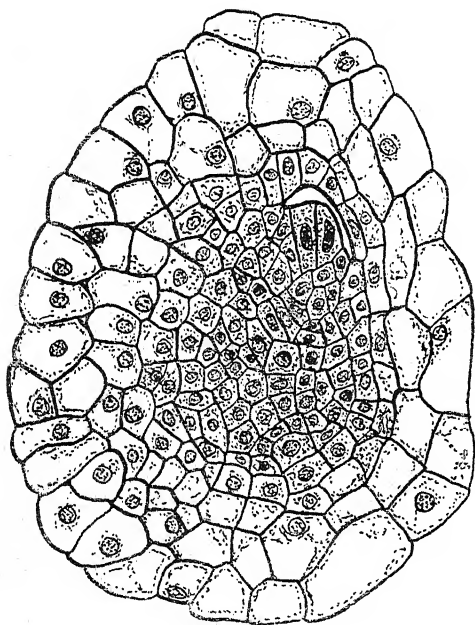
nation of the leaves of successive segments, which is so characteristic a feature of *Equisetum*, necessitates the development of a connecting system at the nodes. In the youngest nodes no such linking up is at first apparent, but provision is soon made for a continuous conducting system by the specialization of certain of the parenchymatous cells of the nodes (Plate VII, Figs. 3, 4). These cells develop into short obliquely placed reticulate tracheides, so arranged as to connect up the strands of successive internodes (Text-fig. 4, and Pl. VI, Fig. 7). This nodal structure is a most characteristic feature of

the genus, and has also been shown to occur in all the stems of *Calamites*; it may therefore be considered as a relatively primitive character, since it is common to both living and fossil representatives.

#### ORIGIN OF THE SECONDARY AXIS.

Provision for the first branching is made very early in the history of the primary shoot, even while the latter is still enclosed within the folds of the prothallus.

Hofmeister (3), in his classical account of Vascular Cryptogams, figures a young sporophyte with the first lateral bud pushing its way through the tissues of the mother axis. He undoubtedly considered this bud to have an endogenous origin, but did not comment upon it in particular because at that time all the lateral buds of *Equisetum* were thought to arise from the central tissues.



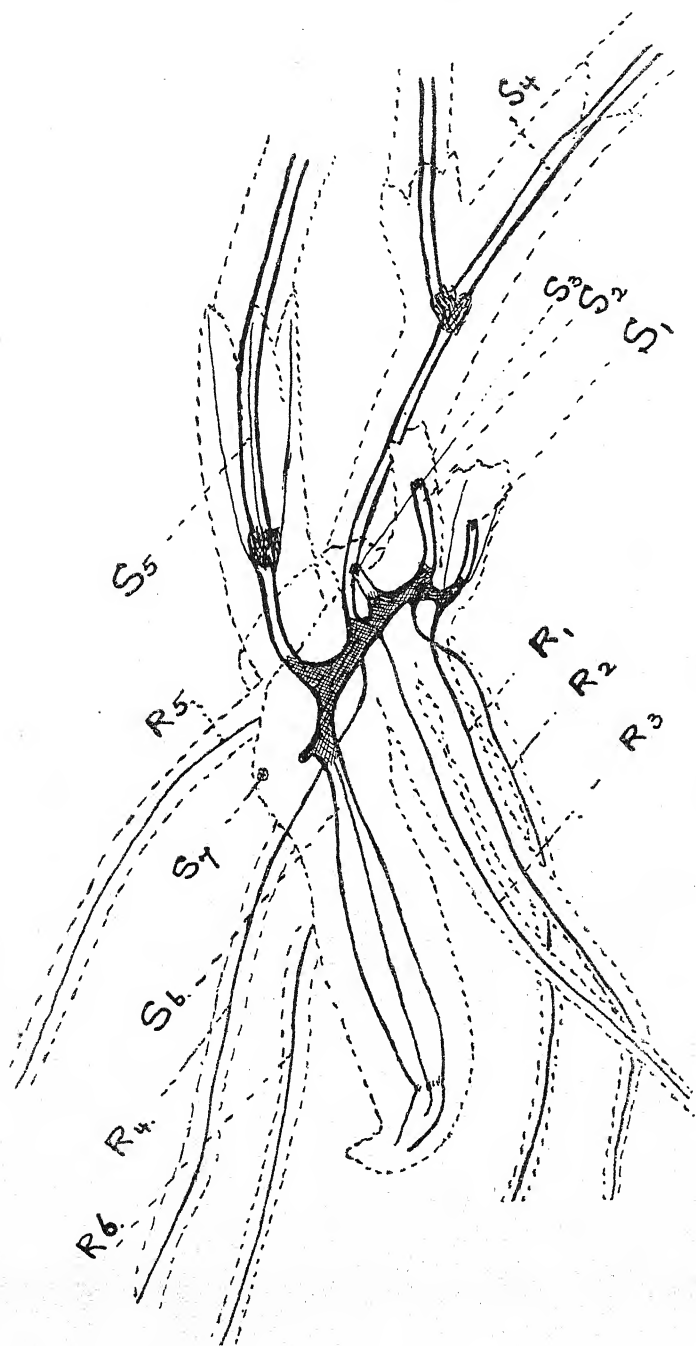
TEXT-FIG. 5. Transverse section from base of primary axis showing origin of the first bud. The apical cell is already established, although the xylem of the primary stem is not yet differentiated.  $\times 950$ .

It was later shown by Janczewski (8) and Vidal (6) that the whorled aerial branches take their origin from buds which arise from superficial cells of the stem at the base of the leaf-sheaths. It was consequently assumed that all the lateral buds arose in like manner.

Jeffrey (4), however, in his description of the sporophyte, refers to its position low down

on the axis well below the leaf-sheath, and speaks of it as the 'so-called adventitious bud', but does not make it clear whether this expression refers to its position on the axis or to its endogenous origin.

In order to determine the latter question, sporophytes were sectioned in various directions. It was found that the bud initial is formed at a very early stage in the history of the primary shoot, in fact as soon as the first leaf-sheath is organized. From an examination of Text-fig. 5, one of a series of transverse sections, it is seen that the bud is undoubtedly endogenous and probably originates in the layer which gives rise to the endodermis. The young branch develops slowly and gradually pushes its



TEXT-FIG. 6. Diagram of symphydial underground stem system of a sporeling. The outlines of the organs are represented by broken lines and the vascular strands by continuous lines. The shoots are numbered (S1-7) according to order of formation. Similarly, the corresponding roots are labelled (R1-6). x 20.

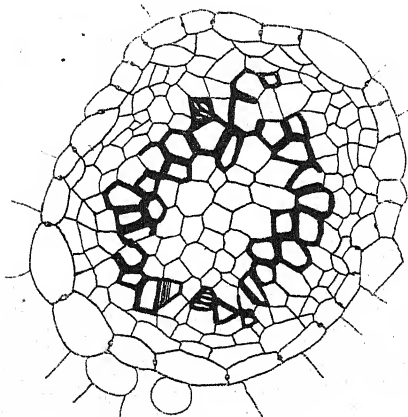


way through the tissues of the parent axis. It behaves in fact like a young root, from which, of course, it is early distinguishable by the development of its first leaf-sheath.

The vascular tissues originate in the bud quite independently of the mother axis. The first indications of a connexion between the parent and lateral shoot appear next to the stele of the parent stem, by the differentiation of a hollow tube composed entirely of short wide tracheides with reticulate markings (Text-fig. 2).

The lateral shoot bears at its base a sheath formed from the fused bases of the first whorl of leaves, which is commonly spoken of as the ochreola. The leaves of this whorl have no vascular strands, as Jeffrey (4) observed; the nodal tracheides make their appearance, however, in the

appropriate position, and, by the differentiation below them of other short tracheides, are connected to the vascular tube already described, which is thus extended to the level of the insertion of the ochreola (Plate VII, Fig. 2). The absence of vascular tissue from the ochreola of this the first lateral branch of the young plant is a feature common to all the succeeding branches, wherever they may be produced. The leaves of the first whorl of every branch, whether arising from the rhizomes or from the aerial shoots, have no leaf-traces. On the other hand, it may be recalled that the



TEXT-FIG. 7. Transverse section of base of secondary axis, showing siphonostele.  $\times 275$ .

leaves of the first whorl of the primary axis do possess a vascular supply, but there are indications that it is gradually being suppressed. This is notably the case in the leaf adjoining the foot, where the trace is often only partially formed and is frequently absent altogether, the only indication being a protrusion of the endodermis (Text-fig. 2).

At the level of the ochreola, or a little below it, a third shoot takes its origin from a bud on the second axis and develops in a similar way to that already described. This bud appears early and grows out through the tissues of the parent shoot, and by continuing in a horizontal direction for a short distance before turning up above ground it adds its contribution to the underground axis. The first aerial shoots are all formed in this way, and as a result a short sympodium is built up from their bases (Text-fig. 6).

The structure of the xylem throughout this region consists of a tube of reticulate tracheides interrupted at intervals by the attachment of the

vascular supplies from the aerial shoots (Text-fig. 7). There are no spiral and annular elements present, and this may be accounted for by the absence of elongation in this region of the axis once the conducting tissue has developed. This underground region thus presents features which only appear in a modified form at the bases of the ordinary aerial axes. As has been shown above, these structural peculiarities can be directly attributed to the early and repeated branching by which it is built up.

#### ORIGIN OF THE METAXYLEM.

The structure of the bundles in the internode of the mature stem is too well known to need detailed description. Of the three groups of xylem normally present, the two lateral groups, commonly known as the metaxylem, develop later than the elements of the protoxylem which are associated with the carinal canal.

There have been two different interpretations of the nature of the metaxylem of the internodal bundle. The earlier investigators concluded that it comprised the laterally developed elements of a collateral bundle of which the first-formed elements were represented by the protoxylem adhering to the carinal canals. The similarity in the general arrangement of the tissues as seen in transverse sections of the internodes of any *Equisetum* compared with that of a young herbaceous dicotyledon naturally gave rise to the opinion that the structures were analogous. Such a type of structure is, however, unusual in Vascular Cryptogams apart from the well-known case of the Osmundaceae. In all the groups of Vascular Cryptogams other than the Equisetaceae the mature structure, even in the most elaborate types, can be shown to have been derived, in the course of ontogeny, from a simple protostele.

In 1901 Prof. Gwynne-Vaughan (9) suggested another interpretation for the metaxylem. He pointed out its independent nature and showed that the protoxylem of the carinal canals was alone associated with the leaf-trace system, and he concluded, at least for *E. hiemale* and *E. maximum*, that the metaxylem strands were continuous over the nodes, merely altering their position laterally and becoming associated with neighbouring leaf-traces. He went farther and suggested tentatively that this xylem might represent the last remnants of a mass of centripetal wood of a primitive protostele. This would involve the centripetal development of this portion of the wood, and he supported his theory by reference to *E. giganteum*, which showed what he interpreted as indications of such a development, but owing to the mature condition of all the available material he could not demonstrate this conclusively.

The question has thus definitely centred upon the order of differentiation of this metaxylem. Eames (10) and Quéva (7) have subsequently stated that the whole of the lateral metaxylem is centrifugal, although the

former records examples of certain irregularities in the direction of its lignification.

With regard to the elements composing this metaxylem, Quéva (7), from an examination of *E. maximum* and *E. limosum*, considers that they are all spirally thickened tracheides; but Eames (10) mentions that in *E. maximum* the elements of the lateral strands generally show scalariform, or reticulately pitted walls with occasional annular and spiral thickenings.

According to the results of the present investigation, there is no question that in *E. arvense* the majority of the tracheides composing this metaxylem are spirally thickened, the first developed ones being considerably drawn out during the later stages of elongation, and in some cases the elements break down, leaving small lacunae comparable to those associated with the protoxylem. On the other hand, in the later-formed elements it is seen that the thickening is more closely disposed until a simple form of reticulum results, requiring careful examination to distinguish it from the closer spiral forms.

This is found most elaborately developed in *E. giganteum*, and confirms Gwynne-Vaughan's observation that the smaller size of the outer elements suggested a centripetal order of development.

The order of differentiation of this particular region of the vascular system has been carefully traced both in the young sporeling and in the apices of adult shoots belonging to the following species: *E. arvense* and *E. maximum*. Although there is a variation in the amount of xylem developed, the order of its appearance is fairly constant.

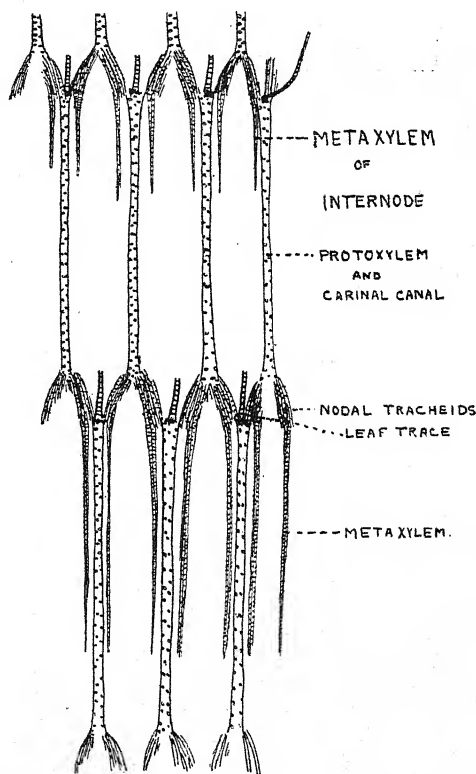
The tissues which are destined to form these lateral xylem elements are early distinguishable as procambial strands. Elongation of the elements is almost completed in the internodes before any thickening takes place, although in some cases where growth has continued after the completion of the tracheides a certain amount of disruption occurs with the formation of small lateral lacunae. The thickening and lignification of the tracheides proceeds regularly through the internode; beginning in one cell which abuts upon the nodal tracheides, the process follows in the cells immediately below until a whole column is lignified (Text-fig. 8 and Pl. VII, Fig. 1). Before this is completed, however, another vertical row of cells begins to differentiate in the same way, and this scheme is followed until the whole group of tracheides is matured. Owing to the fact that the lignification does not take place simultaneously throughout the internode, it is possible to select one in which the material is in a half-developed condition. Some stem apices about two inches long were halved longitudinally, and one half of each apex was cleared with eau de Javelle and stained with fuchsin in the way already described. From these it could be ascertained which nodes showed the desired condition, and the companion halves were embedded in wax and microtomed. From the two sets of preparations it was possible to

determine the order of differentiation of the elements in the lateral bundles.

It was thus clearly made out that the spiral and annular elements occur nearest to the carinal protoxylem, and that those subsequently differentiated occur nearer to the periphery of the stem and more remote from the canal.

The metaxylem strands of successive vegetative internodes are not directly continuous, but are linked together by means of the short, coarsely reticulate nodal tracheides, and no indication was found of any of these strands crossing the outer surface of the nodal xylem, as has been suggested by Gwynne-Vaughan (9) for *E. hiemale* and *E. maximum*. The direct relation between the carinal protoxylem and the lateral metaxylem in the internode of the aerial shoots is not very clear owing to the separation in space and time between their respective developments. Further reference will be made to this, however, when the structure of the tubers and cones has been described.

In the course of an examination of some young vegetative axes of *E. maximum*, the large nodal tracheides described and figured by Sykes (11) were met with. They form quite early in the life of the shoot, and are clearly ordinary nodal tracheides which owe their shape to the opportunity of free development afforded by their projection into the lumen of the canal. They are undoubtedly concerned with the transference of water from the carinal canals of one internode to those of the internode above, as Miss Sykes suggested.



TEXT-FIG. 8. Diagram illustrating late development of the metaxylem. Length of internodes much reduced.

## ORIGIN OF LATERAL BUDS.

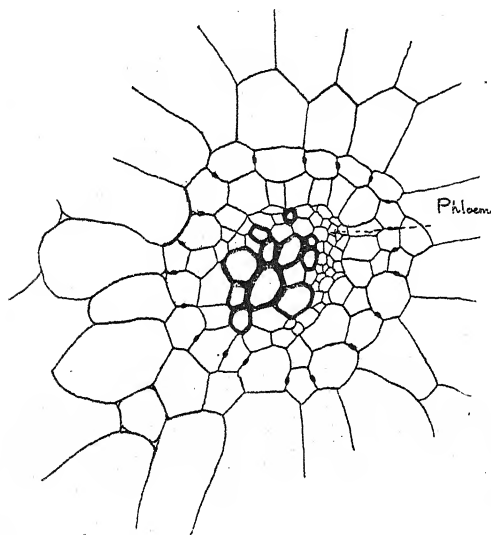
The primary axis only gives rise sparingly to lateral buds, but as the successive aerial axes develop, they produce whorls of branches. These arise from buds which have been clearly demonstrated to be of exogenous origin (6, 8) and to occur in the axil of the leaf-sheaths, alternating in position with the leaf-teeth.

The tracheides of the bud internodes follow exactly the same order of development as those of the primary axis, i.e. the internodal elements become lignified first and are linked up with the leaf-traces and with the

corresponding elements of higher internodes later by the short tracheides of the node.

The junction of the bud vascular system with that of the parent stem is brought about by the formation of a closed tube of short, strongly thickened elements repeating the structure already described which is found at the base of the secondary axis.

*Rhizomes.* The first rhizomes arise as lateral buds at the base of young aerial stems. In *E. arvense* they appear, as a rule, after three or four upright shoots have been formed, but this is dependent upon conditions of growth, as already stated.



TEXT-FIG. 9. Transverse section of a bundle from a normal tuber of *E. arvense*.  $\times 275$ .

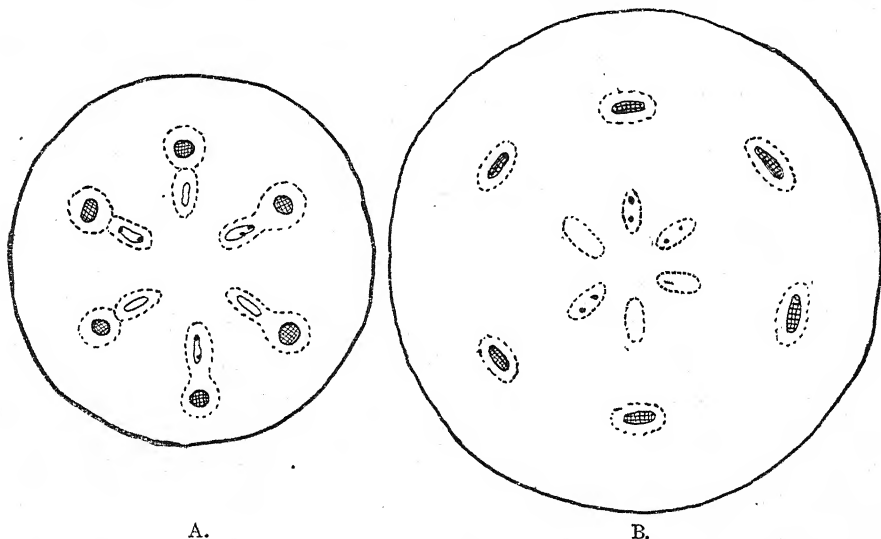
A rhizome is like an aerial shoot except with regard to its direction of growth and its lack of chlorenchyma. Where the plants are left crowded together these rhizomes have not so good a chance of developing, and their growth may consequently be arrested. Towards the end of the summer other shoots grow out as short fat tubers (Pl. VI, Fig. 1, *e, f*). These *tubers* in the young plant consist only of one long swollen internode crowned by a small node and terminal bud, although in older plants a chain of tubers may be formed by the development of successive internodes.

There is a striking difference in structure between these two kinds of underground organs, both of which often grow out as lateral buds from the same axis at the same node, and apparently under the same conditions. The main difference in structure, apart from the great increase in paren-

chyma, is a complete absence of air-channels in the tuber. In the tubers of the sporelings there are usually four bundles embedded in a great mass of starch-containing parenchyma. Each vascular strand is surrounded by a very evident endodermis, and in this respect the tuber of *E. arvense* differs from any of the other shoots of this species (Text-fig. 9).

Tubers which are formed on the rhizomes of mature plants vary considerably in form, as has been fully described by Duval-Jouve (12) and Milde (13). They may consist of single swollen internodes, or of a series similar to the well-known case of *Arrhenatherum avenaceum*.

In the more swollen tubers, as Leclerc du Sablon (14) has shown, the vascular strands branch before reaching the greatest diameter and link up

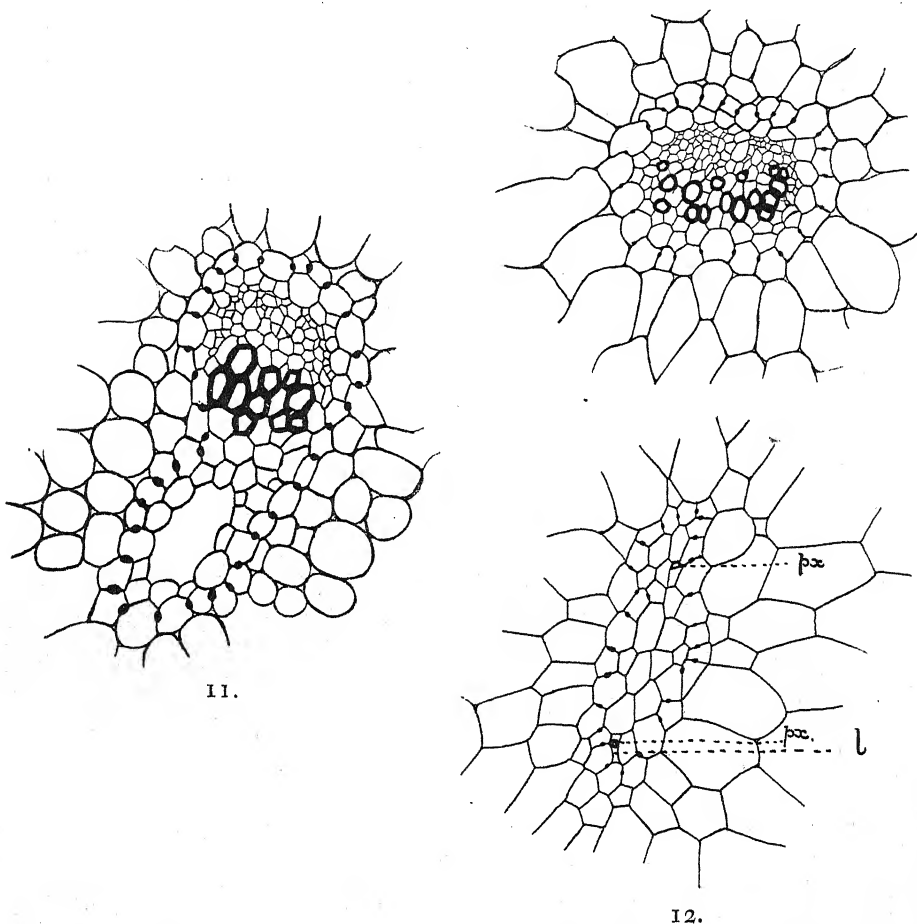


TEXT-FIG. 10. Diagram of transverse sections of abnormal tuber. A. Close below a node. B. Middle of the internode.

again before passing through the nodal region. The same author has pointed out that each vascular bundle is made up of mixed tracheides and parenchyma, and the xylem does not show a separation into three parallel strands.

Among the material examined during the present investigation one tuber of *E. arvense* was met with which exhibited a distinctly anomalous structure. It was noticed that in transverse section the bundles appeared to be much elongated in the radial direction, and on closer inspection it was found that the protoxylem, consisting of one or two extremely narrow elements, was separated from the remaining xylem by a group of parenchymatous cells, the endodermis encircling the whole bundle (Text-figs. 10 (A, B), 11, and 12). A series of sections was taken through the remainder of the internode, and from this it was seen that towards the widest region of the

tuber these protoxylem elements remained relatively in their original place, while the remaining elements of the bundle occupied a more peripheral position. As the separation became more extreme, one endodermis no longer surrounded both parts, but separate ones encircled each portion of xylem (Text-figs. 10 (B) and 12).



TEXT-FIG. 11. Transverse section of a vascular bundle of an anomalous tuber of *E. arvense* close below a node. The carinal canal is clearly visible and is enclosed with the remainder of the bundle by a common endodermis.

TEXT-FIG. 12. Transverse section of a bundle from the middle of the same internode of anomalous tuber. The protoxylem is separated by a considerable radial distance from the metaxylem, and is enclosed in a separate endodermal sheath. Much of the parenchyma separating the two bundles in the radial direction has been omitted. *px* = protoxylem; *l* = lacuna.  $\times 180$ .

The relatively small number and small size of the protoxylem elements and their separate position in this anomalous tuber thus suggest that the bulk of the tracheides present in the normal bundles represent the counterpart of the metaxylem of the aerial shoots and cone axis.

A considerable amount of material was looked over in order to find other specimens with a similar structure, but without success. It seems clear, however, from this one case that the xylem in the bundle of the normal tuber consists of a relatively large mass of tracheides comparable to the lateral strands of metaxylem found in the typical vegetative internodes, while the protoxylem associated with the carinal canals of the latter is usually indistinguishable in the mature tubers. It is not suggested that this is not present at all, but if formed is there in very small amount, and owing to the tremendous development of parenchyma and consequent radial extension the small canal formed by its disruption becomes obliterated and the remains of the elements are no longer visible. It has already been mentioned that the protoxylem elements of the abnormal tuber are extremely small, and would probably have been overlooked had it not been for their separation from the main elements of the bundle and their inclusion within a separate endodermis.

The full significance of this structure will be evident when compared with the cone and vegetative axis.

#### SECONDARY THICKENING.

Since the appearance of Cormack's (15) observations on the xylem of *E. maximum*, the view has been very generally held that a limited amount of secondary growth takes place in the xylem of the nodes of certain species of *Equisetum*. Cormack (15) supported his conclusions by a comparison between the number of elements present in the nodal groups of xylem at the apex and in the same relative position several internodes lower in the stem. He shows that in the successive nodes the number of elements of lignified tissue increases, whereas the number of elements of the internodes remains more or less constant. This observation, which is readily confirmed, does not necessarily prove the point, for the increase in number of elements as seen in transverse section may be due to other causes than multiplication by cambial cell division. Longitudinal sections were examined in order to determine whether this 'increase' at any particular level had not been produced by the elongation of the elements in question accompanied by displacement due to sliding growth.

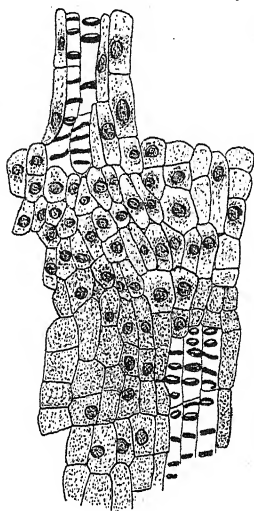
To determine whether this has actually happened, it is necessary to measure the lengths of the elements in the various regions and to examine critically the relation existing between the cells of the nodes and the internodes. In *E. arvense*, some dormant underground buds were fixed and sectioned in a longitudinal direction. These buds were particularly favourable for such an investigation because the numerous nodes in each bud showed every stage in the development of the xylem.

Similar buds of *E. maximum* were also examined and the same conditions were found. The drawings and measurements were, however, made





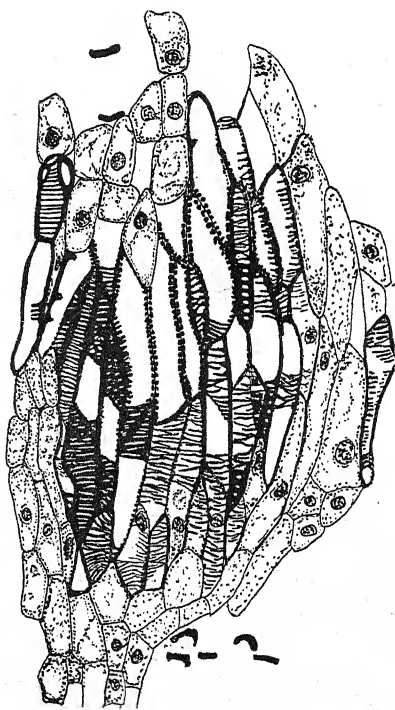
13.



14.



15.



16.

TEXT-FIGS. 13-16. Cells of the node of a vegetative bud of *E. arvense* seen in longitudinal tangential view, showing four stages in the development of the nodal tracheides.  $\times 275$ .

from specimens of *E. arvense*, owing to the more convenient size and shape of the buds.

Text-figs. 13-16 show groups of nodal elements drawn from the nodes of an apical bud. From these it will be seen that the shape of the elements gradually changes from short cubical cells to slightly elongated elements tapering at both ends. The first change involves a slight lengthening of each element. Since little or no growth is taking place in the surrounding cells, the elongation of these xylem elements necessitates some special accommodation. This is brought about by an overlapping of the ends of the cells and an inclination of their long axes. All the elements of a group have the same inclination, though the direction is reversed in contiguous bundles, as seen in tangential sections. As the elongation of these elements proceeds, it is accompanied by an increase in diameter, and finally the walls are thickened in a reticulate pattern and lignification completes the development.

The cells which are destined to compose the nodal regions of the stem are sharply marked off from the internodal elements, which multiply rapidly later. The former are cubical parenchymatous cells arranged in very symmetrical rows, about four tiers of such cells being concerned in the formation of each node.

When the tracheides are fully grown, but before they lose their protoplasmic contents, the position of the nuclei still gives a rough indication of the original arrangement of the cells. If these groups of cells were sectioned in a transverse direction it would naturally appear as if the number of elements had increased as the node became older. This increase, however, is brought about by the *overlapping* of previously existing cells, and cannot in any way be referred to a process of secondary thickening. Each element in longitudinal view is seen to overlap one—two cells, which would result in an apparent multiplication by two or three, and on counting the number of elements present in a number of groups as seen in transverse section the average increase corresponded very closely, i.e. rather more than two.

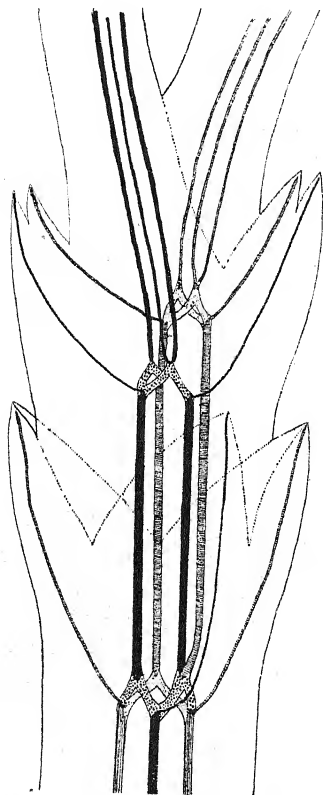
The examination of preparations of *E. maximum* lead to the same conclusions, and it would seem, therefore, that in these two species the facts on which the existence of a process of secondary growth has been based are susceptible of a simpler explanation, although one which perhaps will not be so acceptable to palaeontologists.

#### A FORKED VEGETATIVE AXIS.

Several authors have already described cases of branching in cones of *Equisetum*, and Stiles (16) has investigated the anatomy of a branched strobilus of *E. maximum*. He accepts the view put forward by Bower (17, p. 682) that the branching of the strobilus is fundamentally dichotomous. It is interesting, therefore, to be able to record an example of an apparent

dichotomy of a vegetative axis. One of the sporelings of *E. limosum*, grown in culture, was found to have a forked primary axis. Only one such case was seen, the forking of the axis taking place at the level of the fifth node. The specimen was cleared and stained with ammoniacal fuchsin so that the vascular strands stood out clearly.

It may be seen by reference to Text-fig. 17, that the vascular strands of the two branches run for a short distance as separate systems in the partially fused stem, and two nodes with the intervening internode are involved in the adjustments of the vascular system. The changes so necessitated include a reduction of six strands to three, and this takes place in two stages. The normal number of leaves for the sporeling is three, but at the node near the point of forking four appendages are present. The vascular system of the two branches shows greater independence at this region than do the external tissues; there are in fact two distinct vascular cylinders, and a separate nodal organization is present for each branch. Each of these supplies traces to a pair of leaves.



TEXT-FIG. 17. Diagram illustrating the vascular system of the sporeling of *E. limosum* with the forked axis. The difference in level of the two nodes in the common axis is real and not due entirely to perspective.

In each case the three bundles of the branch are continued by two in the internode below. The reduction is effected by two bundles approximating immediately above the node and linking up with the same group of nodal tracheides. At the node below the four strands, two from each branch are similarly replaced by three in the next internode. It may be pointed out that this simple method of varying the number of bundles in adjoining internodes is that normally followed in ordinary shoots.

It is evident from the facts described above that this is not a case of vigorous development of a lateral branch, but a true forking of the primary axis.

#### THE STRUCTURE OF THE CONE.

A careful and detailed account of the structure of the cone of four species of *Equisetum* has been given by Lady Isabel Browne (1, 2). Cones of each of these species were cut into series of transverse and longitudinal

sections, from which diagrams of the whole vascular system were constructed.

Browne concludes that the species examined can be arranged in a series which shows a progressive reduction in the amount of xylem tissue. This ranges from a regular arrangement in *E. arvense*, imperfectly siphonostelic at the nodes to dictyostelic in the internodes, through *E. palustre*, *E. maximum*, to *E. limosum*, where the xylem is reduced to an irregular network. This reduction in the xylem is accompanied by an increase in size of the parenchymatous meshes which separate the bundles, and in *E. palustre* these meshes often extend through the nodes, with the result that the vascular strands tend to run longitudinally with few anastomoses and are separated by relatively wide parenchymatous tracks.

Browne describes the detailed nature of the vascular bundles, and draws attention to the difference between those present in the strobilus and those occurring in the vegetative axes. She considers that the anatomy of the cone axis supports the view that the sporangiophores are whole appendages of a foliar nature, and interprets the structure of the cone in terms of nodes and internodes. Browne agrees with most authors in regarding the annulus as a modified leaf-whorl.

In the present investigation cones have been prepared in the way already described for the vegetative organs: they were cut in half longitudinally, cleared, and stained in bulk. By this method a large number of specimens of different species could be prepared in a relatively short time and the individual variability appreciated. Both very young and mature cones were thus prepared in order to trace out the ontogeny of the vascular system.

The first impression gained from an examination of the cone is the marked differences in structure it presents as compared with the vegetative axis. Of these the most striking are the apparent absence of lacunae, both vallecular and carinal, and the structure of the vascular bundles.

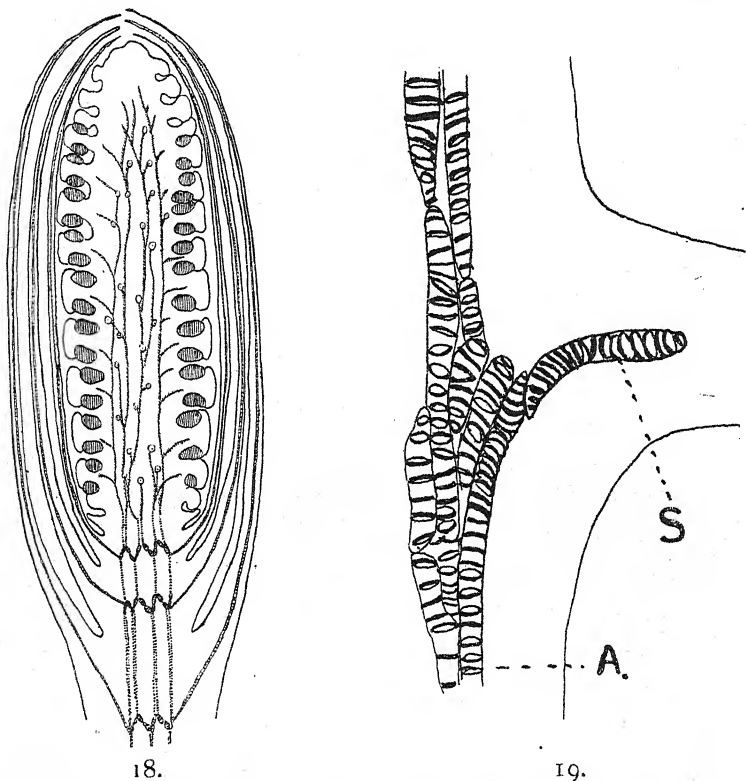
Browne (1, p. 684) has pointed out that instead of the three groups of xylem, characteristic of the bundles of the vegetative internodes, the bundles of the cone show a narrow tangentially extended band of tracheides, and suggests that 'in the vegetative internodes the lateral groups of xylem represent the free ends of a more deeply curved band of xylem, and in that case the position of the tracheides of the lateral groups of metaxylem in a radial series is due to the more or less marked curvature of a band of which only the carinal tracheides and the free ends are lignified. The primitive form of the internodal bundle would then be that of the cone.'

She further states (1, p. 666): '... In a good many places there are, internally to the bands or ring, isolated tracheides or little groups of tracheides, usually of small size; ... Such tracheides or groups of tracheides do not as a rule persist for any considerable distance in a vertical direction;

in the internode they occur also internally to the separate strands of xylem.' It may be stated quite definitely that these elements belong to the protoxylem, although Browne does not apparently identify them as such, and, as will be shown below, they form a definite system of strands and constitute the scaffolding on which the whole vascular system of the cone is built up.

The species examined were *E. maximum*, *E. arvense*, *E. limosum*, *E. palustre*, *E. sylvaticum*.

In very young cones of *E. palustre*<sup>1</sup> the development of the xylem could be readily made out (Text-fig. 18). It proceeds in a manner in no



TEXT-FIG. 18. Diagram representing half a young cone of *E. palustre*. The sporangiophores and leaves are somewhat diagrammatic, and the small circles surrounding dots in connexion with the vascular strands represent the points of connexion of the sporangiophore traces not otherwise shown. The protoxylem strands were traced with a camera lucida.

TEXT-FIG. 19. Xylem strand of cone and the first differentiated tracheide of the sporangiophoric trace from young cone of *E. palustre*. A = axial strand of cone; S = sporangiophoric trace.

way comparable with that of the vegetative axis. In the latter, the several protoxylem strands of each internode develop simultaneously. Their

<sup>1</sup> Cones of *E. arvense* and *E. maximum* were collected in the autumn and found to be fully formed. As Browne pointed out for *E. arvense*, the vascular system is at this time practically mature. *E. palustre* was therefore selected owing to the fact that the cones are formed at the apices of the vegetative axes, and can be very easily collected at an early stage.

development is quite independent of those in the internodes above and below, with which they are subsequently connected by the nodal tracheides. In the cone, however, the protoxylem strands develop uninterruptedly from the base to the apex of the cone. There is no disjunction at the 'nodes', though some of the strands may terminate at or near the point of insertion of the sporangiophores. Moreover, the traces of the latter arise in a manner quite different from those of the leaves. The differentiation of the tracheides of the leaf-traces begins in the distal part of the leaf and proceeds inwards until they finally link up with the nodal tracheides. In the sporangiophore trace the order of development is reversed. The first tracheide differentiates next to an axial strand of protoxylem, to which it may be connected laterally or terminally, and the further differentiation of the strand proceeds outwards into the stalk of the sporangiophore (Text-fig. 19).

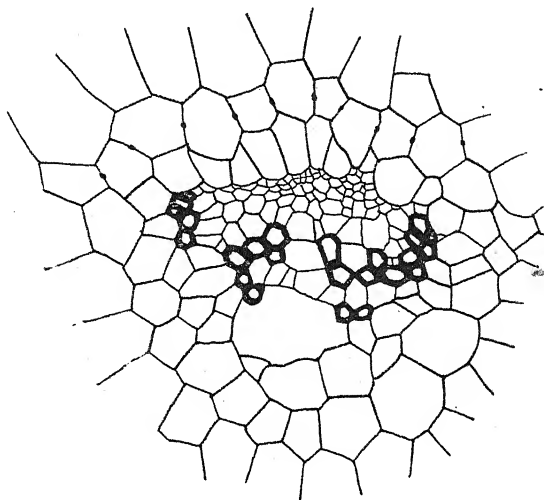
In any comparison of the vascular structure of the cones and vegetative shoots, such marked difference in the arrangement and mode of development of the protoxylem strands must receive full consideration. They are features which would be least affected and longest retained in any process of reduction. In the vegetative shoots the leaf-sheaths, though now of comparatively small importance in the economy of the plant, have a dominating influence on the vascular structure of the stem. The sporangiophores, though provided with a vascular system of relatively better development than that of the leaf-teeth, have no similar effect on the anatomy of the cone.

The examination of mature cones of such species as *E. arvense* and *E. maximum*, with more or less regular whorls of sporangiophores and with well-developed metaxylem masking the protoxylem strands, may perhaps suggest an internal organization of node and internode. A study of the development of the vascular system, however, can only lead to the conclusion that the vascular structure so characteristic of all the mature vegetative axes, with its well-defined segmentation, is quite absent in the cone.

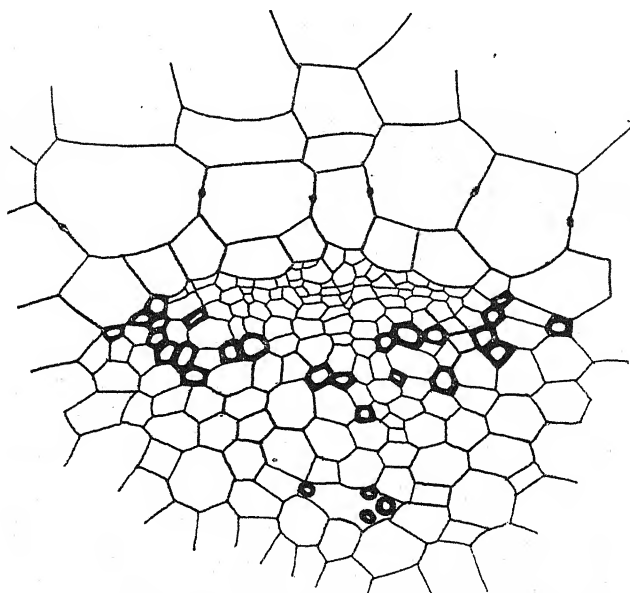
With the appearance of metaxylem tracheides in the axis—which takes place somewhat irregularly—additional elements are added to the sporangiophore traces, which when completed show a mesarch structure as described by Eames (19). The axial metaxylem consists of spirally thickened tracheides which vary considerably in length. The advantage of this type of tracheide is evident in view of the rapid elongation of the cone which takes place at the time of dehiscence of the sporangia.

As seen in transverse sections (Text-figs. 20 and 21), the metaxylem is separated from the protoxylem by parenchymatous cells, and as the cone matures it forms a continuous band, 2 to 4 cells wide, external to the protoxylem in each bundle. The greater development of the metaxylem tends to mask the presence of the protoxylem. The latter, however, is

always present and is most conspicuously seen in transverse section in cones of the shape found in *E. palustre*. There the growth in length is relatively



TEXT-FIG. 20. Transverse section of bundle from cone of *E. palustre*. Note the carinal canal.  $\times 275$ .

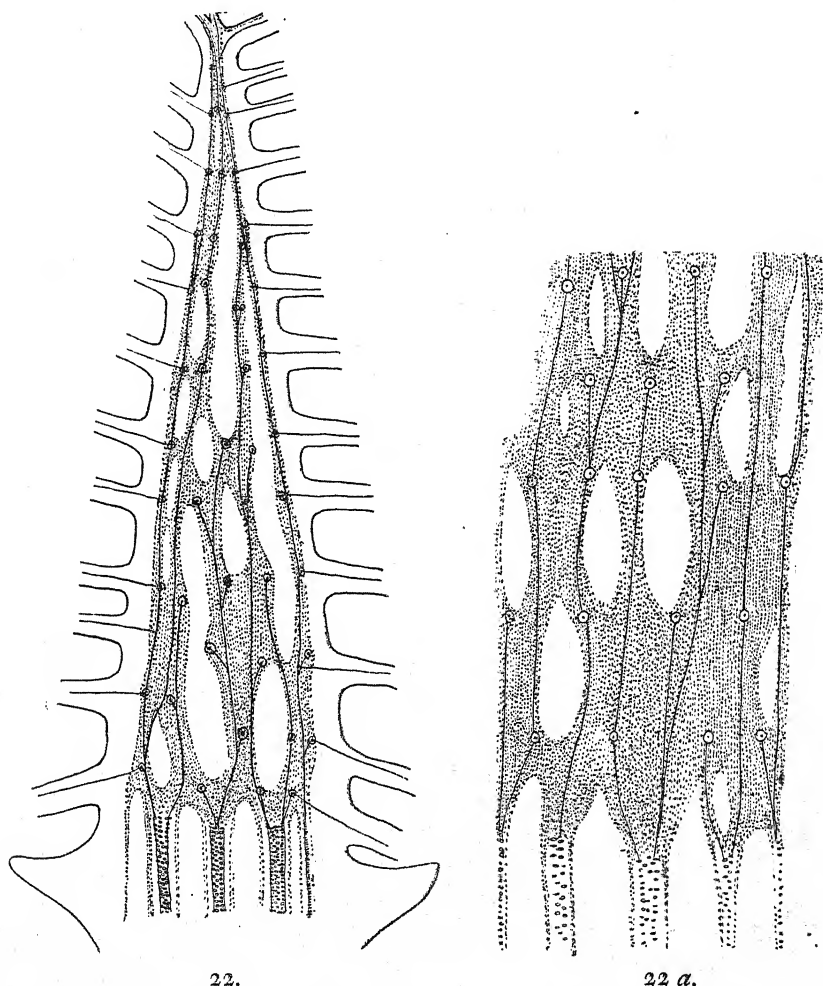


TEXT-FIG. 21. Transverse section of bundle from cone of *E. maximum*. Note the scattered arrangement of the metaxylem and the position of the protoxylem.  $\times 275$ .

great, bringing about a more complete rupture of the protoxylem and consequently the formation of small lacunae (Fig. 20). The relation between

the structure of these bundles and that of the bundles of the vegetative stems will be discussed below.

Although, as already stated, the protoxylem strands determine the main features of the vascular system, yet the very considerable development



22.

22 a.

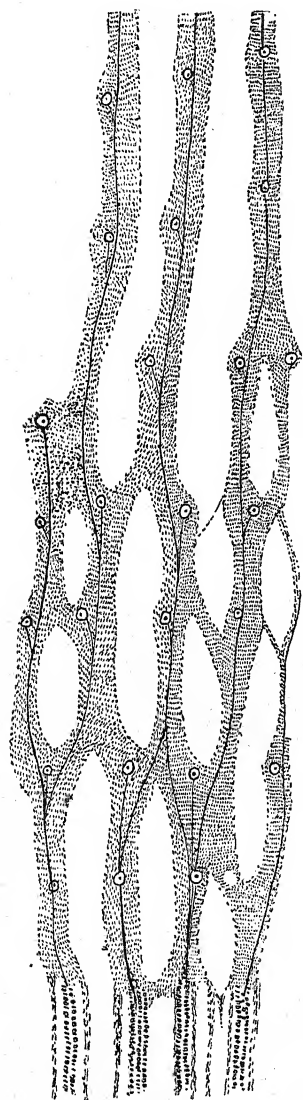
TEXT-FIG. 22. Diagram illustrating the vascular system of the cone of *E. arvense*. The metaxylem is represented by shaded area and the protoxylem by continuous lines. The black circles enclosing central dots represent the point of attachment of the sporangiophore traces.

TEXT-FIG. 22 a. Part of the vascular system of a cone of *E. arvense*, showing more extensive tracts of metaxylem.

of metaxylem in the bundles may tend to obscure the course of the primary strands (Text-figs. 22 and 22 a). Moreover, the metaxylem may be unequally developed on either side of a protoxylem strand, and may in certain regions be so well developed as to form lateral connexions with



neighbouring strands. This is, in fact, what frequently happens at the 'nodes', the anastomosing and forking of the bundles having in many cases no relation to the behaviour of the primary strands, although of course forking



TEXT-FIG. 23. Diagram illustrating the arrangement of the xylem strands of a cone of *E. maximum*.

of the primary strands does occur. Such lateral connexions were observed by Browne (2, p. 258) in the 'internodes' of the cone of *E. maximum*, and dismissed as not being palingenetic, but were regarded as constituting a fresh character in the phylogeny of the genus. Such a description, which the present writer cannot accept, might equally well be applied to much of the metaxylem developed at the 'nodes' (Text-fig. 23). In some cones of *E. arvense* the metaxylem may be so extensively developed as to form broad bands connecting three or four bundles laterally and extending from the insertion of one whorl of sporangiophores to another (Text-fig. 22 a).

The parenchymatous tracts which separate the bundles have been identified with foliar gaps, caused in the first instance by the departure of the sporangiophoric trace. The gap, however, does not occur immediately above the point of departure of the trace; frequently there is no apparent relation between them. Much of Browne's papers are devoted to a careful attempt to explain the numerous anomalies which occur in the examples she describes. This interpretation of the vascular structure of the cone is of course bound up with the morphological nature of the sporangiophore, and the question will be further discussed below.

Although such species as *E. arvense* and *E. maximum* show a definite network of strands with short meshes, in *E. palustre*, *E. limosum*, *E. sylvaticum*, the meshes are more irregular—frequently much elongated—stretching through two or more 'nodes', and in *E. palustre* even extending nearly the whole length of the cone.

Browne (1, p. 699) ascribes these differences to a reduction of the xylem which, operating at the nodes, has produced an 'extension of the parenchymatous meshes upwards, downwards, and laterally.'

There is no question that in certain species the xylem is less well developed than in others, and it is possible to arrange the species as Browne has done in a reduction series. Whatever factor may have been operative in bringing about this reduction of the xylem considered as a whorl, the present writer cannot admit that it has been influential in determining the course and distribution of the xylem strands and the disposition of the parenchymatous gaps.

It is suggested that the determining factor in the relative development of the metaxylem, and hence of the meshes, is primarily a mechanical one. It is significant that the species with large and heavy cones have more abundant xylem and more regularly developed network.

It has been shown above that the arrangement of the primary strands of xylem and the mode of origin of the sporangiophore traces do not lend themselves to an interpretation of the vascular system of the cone as built up, like that of the vegetative stem, of an alternation of node and internode. If, then, the vascular structure of the cone is so strikingly different from that of the leafy shoot, the contention that the sporangiophore is a foliar structure—whether whole or part of a leaf—loses much support. In *Calamostachys*, as Williamson and Scott (18) have shown, there is a remarkable difference in the structure of the axis at the region of insertion of the sterile bracts and at the level of attachment of the sporangiophores. The nodes which bear the bracts show essentially the same character (e.g. the short nodal tracheide) as the vegetative nodes of *Equisetum*; the traces of the sporangiophores, on the other hand, are inserted directly on the axial strands without interrupting them. In this respect they resemble those of the cone of *Equisetum*.

Hickling (19) points out that the so-called 'sporangiophore' or 'fertile nodes' should not be regarded as nodes in the same sense as the true or 'tract nodes'. With that conclusion the present writer must agree, and in extending it to the cone of *Equisetum* concludes that in the latter genus the fertile axis is entirely undifferentiated into node and internode.

Bower (17), after a careful consideration of the available data, has pointed out that the balance of evidence is strongly in favour of the non-phyllome theory of the sporangiophore in *Equisetum*, and the facts brought out in the present investigation lend it still further support.

The annulus has generally been considered to be a foliar structure. Its morphology has been fully discussed by Goebel (20), and abnormal forms approaching foliage leaves on the one hand and sporangiophores on the other have been described by Milde (13), Glück (21), and others.

It is of some interest to consider to what extent this view is supported by the vascular anatomy of the cone.

Just above the level of the insertion of the annulus the characteristic vascular structure of the cone begins and the transition from the typical

internode below is marked by a considerable development of metaxylem at this point. This has been interpreted by Browne as affording indications of the presence of a node. According to Vidal's (6) conception of the vegetative axis of *Equisetum* as being made up of a series of segments each composed of a node with an inferior internode, the fact that there is an undoubted internode immediately below the insertion of the annulus would presuppose the existence of a node above. On the other hand, there are certain facts which negative such a view; these are (1) the course of the protoxylem strands, (2) the total absence of vascular supply to the annulus. The protoxylem strands from the internode pass without any disjunction to the level at which the first sporangiophore traces arise, and hence there is no alternation at the so-called 'node'. There may be apparent forking of the strands, but this is due to the fact that the protoxylem strands in the bundles of the internode below are often double, which, separating as they enter the cone, produce this appearance of forking. This appearance is emphasized by the disposition of the metaxylem at this region, which is similar to that at the so-called nodes from which the sporangiophore traces arise.

The absence of vascular tissue in the annulus has been frequently noted. In itself this is not an insuperable objection to the foliar theory because the ochreola which occurs at the base of all branches always lacks traces.

In this connexion it is interesting to consider certain abnormal annuli found among the material used in this investigation. Fertile annuli with well-developed sporangia were found in both *E. palustre* and *E. sylvaticum*. They varied in form; some were fully peltate (Pl. VI, Fig. 3), others bore sporangia only on the upper surface. Not one of the structures, however, possessed any trace of a vascular system.

On the whole, therefore, though the vascular structure does not support the view of the foliar nature of the annulus, it cannot be said to throw much light on the morphology of this organ.

#### DISCUSSION AND SUMMARY.

In order to arrive at a true conception of the vascular structure of *Equisetum* it is necessary to look at the plant as a whole. Any general explanation or theory put forward must be capable of embracing characters found in all the organs of all the species.

It very often happens that a study of the structure of the early stages of the individual throws light on the phylogenetic history of the genus. Jeffrey (4), in his account of the sporeling of *E. hiemale*, describes the vascular system at the base of the first shoot as siphonostelic, but it is clear from a study of sporelings of *E. arvense* and *E. maximum*, that the vascular cylinder at the base of the first shoot in these species is protostelic. This

condition does not persist for long; it is temporarily disturbed by the attachment of the first lateral branch, and above the first node the solid cylinder opens out at once into the much-reduced structure of the internode. Such a protostelic condition is not met with in the succeeding shoots. A very characteristic and significant vascular formation is found in the compound structure formed from the bases of the first few aerial shoots. This is a continuous sympodial siphonostele uninterrupted by leaf-traces or parenchymatous gaps of any kind. It is also significant that a siphonostele is to be found at the base of every branch, whether borne above or below ground, and that it is repeated at every node of the vegetative shoots.

The internode undoubtedly shows a much reduced vascular development, and we must look for an explanation of its characteristic structure in the plant organs which have suffered less reduction. The structure of the basal regions already referred to above provides a very strong argument for a siphonostelic origin, and there seems to be small reason to doubt that the bundle arrangement now present in the internodes is the final stage in reduction from such a condition.

Gwynne-Vaughan (9) first raised the question as to the true nature of the lateral strands of internodal xylem, and Poirault (22) agreed with him in his conclusion that they had no connexion with the leaf-trace system, but were continuous over the nodes, and that their development was in a centripetal direction.

Gwynne-Vaughan suggested that these strands may represent the last remnants of a central mass of centripetal xylem. This view was seriously attacked by Eames (10), who not only thought that the whole xylem of each bundle made up a unit, but that all parts were involved in the formation of the leaf-traces.

From the present investigation it seems certain that the order of differentiation of the elements of the lateral strands is in general centrifugal, as the majority of the later investigators (Quéva (7), Browne (1)) have agreed. Eames (10), however, seems to have overlooked the fact which Janczewski (8) and Quéva (7) had previously noted, that the xylem of each internode develops quite independently, only linking up subsequently by the development of the nodal tracheides. He, in fact, speaks of the carinal canals as disappearing at the nodes and their place being occupied by large-celled protoxylem. The protoxylem certainly does not traverse the node, and no elements of that region can be described in such terms, since they are all alike in character, agreeing in their short length and in bearing reticulate thickenings.

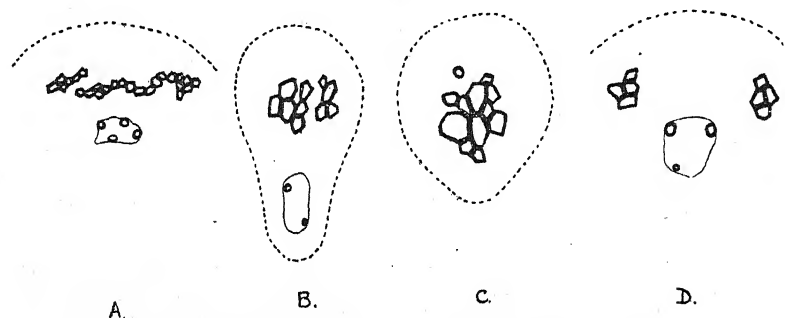
The true nature and relations of these various strands of xylem can be seen by reference to other organs. In the first place the bundles of the tubers give us a clue. There the xylem is not separated into three strands, but forms a continuous group of tracheides, though often interspersed with

parenchymatous cells. The protoxylem is poorly developed and the elements not ruptured, hence a carinal canal is absent. Quéva's figure (7, p. 135) of a bundle from *E. litorale* may be compared with Text-fig. 9.

We obtain, however, still further enlightenment from an examination of the cone.

In the latter organ the predominance of metaxylem is very striking, the protoxylem being represented by relatively few and small elements well to the interior of the irregular bundles. The order of differentiation in the following species, *E. arvense*, *E. maximum*, *E. palustre*, *E. limosum*, *E. sylvaticum*, was undoubtedly centrifugal, but the existence of mesarch traces in the sporangiophores gives credence to Eames's (10) view that the cones of *E. hiemale* and *E. fluviatile* exhibit mesarch axial strands.

Thus the internodal bundles of the aerial vegetative axis are seen to conform in structure with those of the tuber and cone. The lateral strands



TEXT-FIG. 24. Diagram showing bundles from: A, cone; B, abnormal tuber; C, normal tuber; D, internode of stem.

are the relics of a continuous band of metaxylem such as now exists in the bundles of the cone and less perfectly in those of the tuber (Text-fig. 24).

A somewhat similar suggestion was put forward by Browne (1), who, however, regarded the carinal tracheides as representing the middle portion of the once continuous xylem. It has been shown above that in all cases the protoxylem forms an independent strand always internal to the metaxylem.

As Browne suggests, the vascular structure of the cones of some species shows a reduction series, much more xylem being present in the cones of *E. arvense*, for example, than in those of *E. limosum*. It is quite clear from an examination of the former of these species that the basal plan was a siphonostele in which gaps have arisen owing to the failure of the cells to differentiate as tracheides. On this conception the structure met with in the cone links together the unbroken siphonostele of the basal region of the young plant and the vascular structures of the node and internode of the vegetative axis.

The reason for the extraordinary difference in character between the elements of the node and internode of the latter appears to be due to the difference in amount and rate of growth of those two regions. The internodal condition represents the last stage in a reduction of xylem consistent with a continuous and effective supply of water to the leaves and branches.

In the light of these facts it seems clear that the metaxylem found in the underground basal region of the sporophyte, in the cone, the nodes and internodes of the vegetative branches, is all phylogenetically the same, i. e. it is the centrifugally developed wood of a monostelic system, differing only in the degree of reduction and the character of the elements.

With regard to the question of the addition of secondary elements to the centrifugal wood of the node of certain species, the results of the present investigation negative the presence of any process of secondary thickening. The facts on which this idea was based allow of a simpler explanation, viz. that the increase in length of the nodal elements, accompanied by sliding growth, gives an appearance in transverse section of an apparent increase in the number of tracheides of the xylem.

Having concluded that the existing structure in both vegetative and reproductive axes has been derived from a continuous siphonostele only interrupted by the insertion of the branches, we are faced with the problem of the determining factor associated with the distribution of the parenchymatous tracts. Can it be met by an application of the conception of foliar gaps which is associated with the study of filicinean anatomy? Browne has attempted such an explanation in her papers dealing with the cones of several species of *Equisetum*. She homologizes the parenchymatous meshes with foliar gaps, associating them with sporangiophores, and considers that these organs are modified whole foliar structures.

As stated more fully above, the detailed anatomy of the cone does not support the view that the sporangiophores are really of this nature, and, judged in the light of the anatomy of a mixed strobilus such as that of *Calamostachys*, it cannot be doubted that nodes are completely absent in the cones of present-day *Equiseta*.

Even if the sporangiophores were foliar structures, the anatomy does not favour the view that the meshes are foliar gaps. Jeffrey has pointed out, and it is abundantly confirmed in this investigation, that the gap does not, except in a few cases, occur immediately above the point of departure of the trace; and most frequently shows no relation to it.

The theory put forward by Jeffrey (5), that the gaps in the vegetative axis can be described as ramular gaps, is certainly not supported by the anatomical facts described in this paper. The gaps are not situated immediately above the point of insertion of the vascular supply of the branch; they fail, in fact, to satisfy the criterion on which Jeffrey himself insists in the case of foliar gaps.

Whatever factors have been concerned in effecting the gradual lack of differentiation of wood recognizable as such, the actual areas of the xylem in which the replacement of tracheides by parenchyma has taken place have had no necessary relation to the insertion of leaves and branches. The present distribution of the xylem, and hence of the parenchymatous tracts, is such as to best preserve the efficiency of the wood in its double function of water-conduction and mechanical support. In the internodes of the vegetative axis, where the reduction of xylem has been carried very far, it has no doubt made possible the great elongation which is such a marked characteristic of the genus. The rigid tube of short tracheides at the nodes is an important factor in maintaining the mechanical efficiency of the stem. In the cones where the nodes are absent the greater development of meta-xylem and its more equal distribution is adapted to provide for the support of the numerous sporangiophores.

In conclusion, it may be stated that in the writer's opinion the size and distribution of the tracts of parenchyma have no morphological value in the discussion of questions of phylogeny. The same may be said of that much-debated tissue the endodermis. In *Equisetum* not only does it appear in various relations to the vascular tissues in different parts of the same plant, but its arrangement also varies in the corresponding organs of different species. Kashyap (23) has shown in *E. debile* that small groups of parenchymatous cells without any vascular elements may be surrounded by separate endoderms. The anomalous tuber described in the present paper may also be instanced.

It may be recalled in this connexion that similar cases of unusual and independent occurrence of an endodermal layer have been recorded in other plants, e.g. in the roots of *Ruscus* (Lewis, 24).

Whatever factors may have determined its distribution, they are probably physiological ones, which, however, can scarcely be satisfactorily elucidated in the present state of our knowledge.

#### SUMMARY OF RESULTS.

1. The sporeling of *Equisetum arvense* shows a protostelic condition at its base which opens to a siphonostele at the level of attachment of the vascular supply of the secondary axis. The protostelic condition is again resumed for a short distance below the level of the attachment of the first whorl of leaves.

2. The basal regions of the succeeding axes of the young plant possess a compact closed siphonostele composed of short reticulate tracheides. There is thus formed a sympodial vascular tube in which five or more axes may be concerned.

3. The secondary axis arises endogenously from the primary axis below the level of the first leaf-whorl.



4. The vascular structure of an anomalous tuber is described, in which carinal canals are formed in connexion with the protoxylem, and these in the middle region of the tuber are enveloped by separate endodermes.

5. A young sporeling of *E. limosum* is described showing a forked primary axis. The arrangement of the vascular system indicates that it has almost certainly arisen by a dichotomy.

6. The question of the existence of secondary thickening at the nodes of *E. arvense* and *E. maximum* has been investigated by a study of the development of the nodal tracheides. The conclusion is formed that the apparent increase in elements which has been attributed to secondary thickening is due to the enlargement and displacement of developing tracheides.

7. The vascular structure of the cones of *E. arvense*, *E. maximum*, *E. palustre*, *E. limosum*, and *E. sylvaticum* is described.

The endogenous protoxylem strands are shown to form complete and continuous systems, uninterrupted by nodal tracheides, as is invariably the case in vegetative shoots. The metaxylem develops later and varies in amount and distribution in the different species.

*E. arvense* shows the greatest amount and *E. limosum* and *E. sylvaticum* the least.

It is concluded that the gaps in the metaxylem siphonostele cannot be described as leaf-gaps, bearing no relation to the sporangiophore traces, but may be related to the mechanical efficiency of the cone.

It is also concluded that the vascular structure of the cone indicates that the sporangiophores are not the morphological equivalent of leaves, but are organs *sui generis*, and the axis of the cone is undifferentiated into nodes and internodes.

8. The general vascular system of the plant is discussed, and it is concluded that the general plan of development proceeds from a simple proto-tele which opens out into a siphonostele. This shows a considerable reduction in the cone by the development of large parenchymatous meshes or longitudinal tracks and still further reduction in the internodes of the vegetative shoots.

In conclusion, the author's grateful thanks are due to Dr. S. Chandler, who very generously handed over some sporeling material and microtome sections of *Equisetum* sp., which he had already prepared.



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## DESCRIPTION OF PLATES VI AND VII.

Illustrating Miss Barratt's paper on the Vascular System of the Genus *Equisetum*.

## PLATE VI.

Fig. 1. a-g. Drawings of sporelings taken from the same culture towards the end of the first season's growth. Note the great variation in size and development of the different plants grown on prothallia from spores sown at the same time. The prothallial lobes have been removed. Natural size.

Fig. 2. *a* and *b*. External view of lower parts of two sporplings, showing the position of the secondary axis. In *b* it appears much nearer to the root than in *a*. The prothallus has been removed.  $\times 68$ . From drawings by Dr. S. Chandler.

Fig. 3. An abnormal annulus of *E. palustre* showing the development of sporangia. Seen from above.

Fig. 4. Longitudinal view of basal region of very young sporpling from a cleared preparation. The cortical tissues have been omitted. The endodermis is conspicuous owing to the cuticularized band staining deeply with ammoniacal fuchsin. Note the difference in level of the two leaf-traces.  $\times 180$ .

Fig. 5. Transverse section of basal region of young sporpling, showing the central cylinder only. Note the curious appearance of the reticulate tracheides in section.  $\times 320$ .

Fig. 6. Tracheides from the basal region of the same sporpling more highly magnified.  $\times 640$ .

## PLATE VII.

Fig. 1. A node and part of an internode of a sporpling of *E. arvense* from a cleared preparation. Note the first development of metaxylem (M).  $\times 425$ .

Fig. 2. Base of a sporpling of *E. arvense*, showing two aerial axes and the early stages in the formation of the third.  $\times 65$ .

F = foot.  $S_1, S_2$  = primary and secondary axes.

B = bud which will form third axis.

X = vascular supply for B.

O = ochreola.

L = first leaf-whorl.

Lt = leaf-trace.

R<sub>1</sub> = xylem strand of first root.

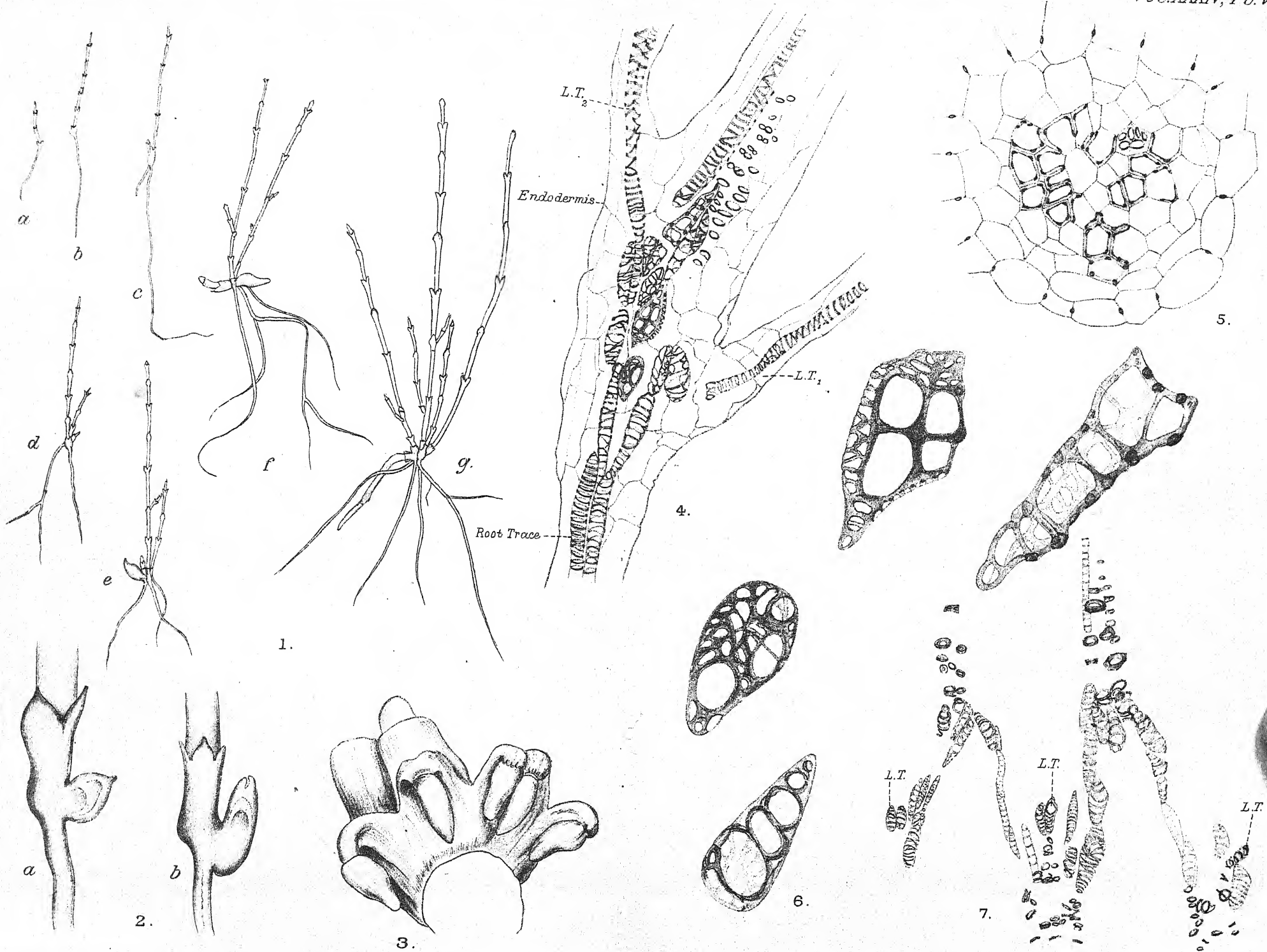
R<sub>2</sub> = xylem strand of second root.

Fig. 3. A young node and internode near the apex of a sporpling of *E. arvense* before the nodal tracheides have linked up the internodal xylem. From a cleared preparation.  $\times 425$ .

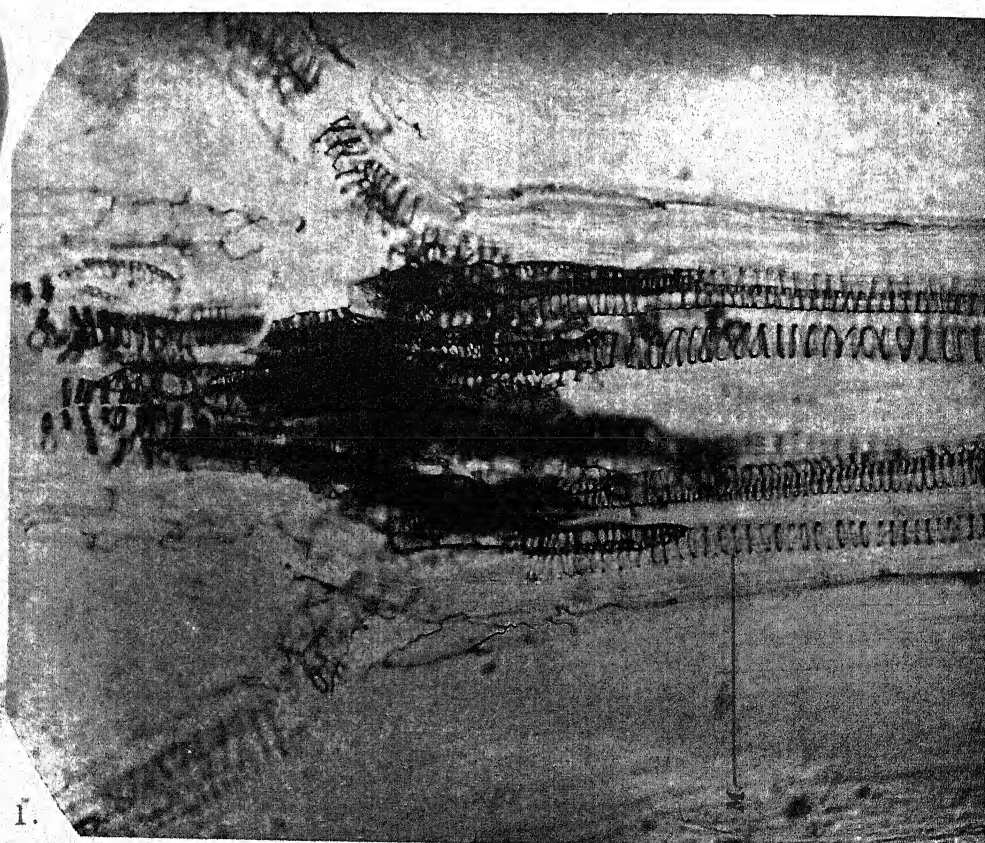
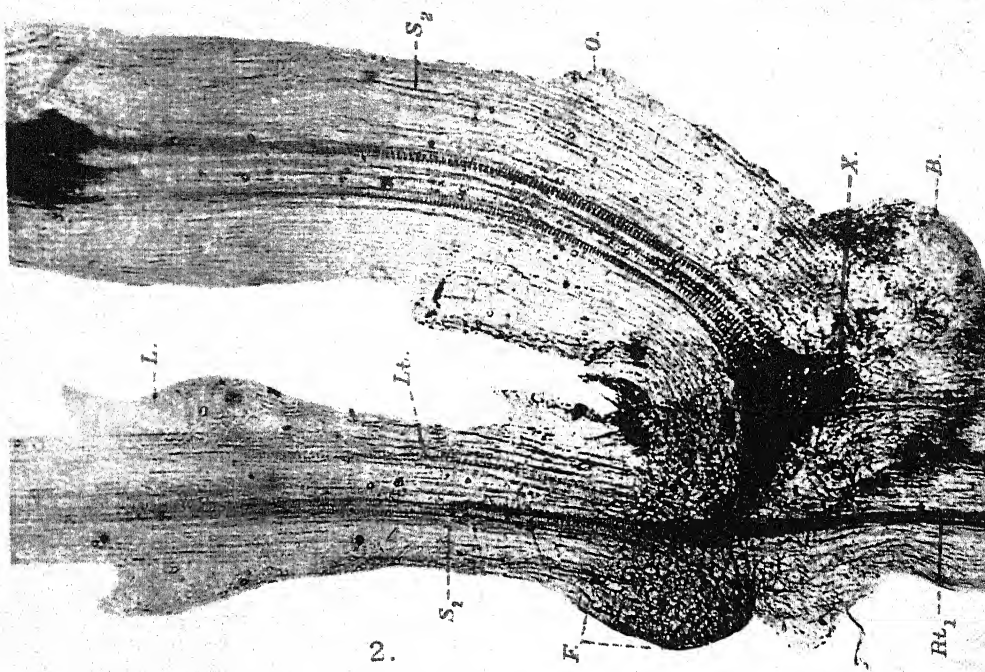
Fig. 4. Four internodes of a bud of *E. maximum*, showing the early differentiation of internodal xylem. Note that the strict alternation of the strands is departed from by the introduction of an extra member in one whorl. From a cleared preparation.

Fig. 5. The vascular supply of half a cone of *E. palustre*. From a cleared preparation.  $\times 12$ .

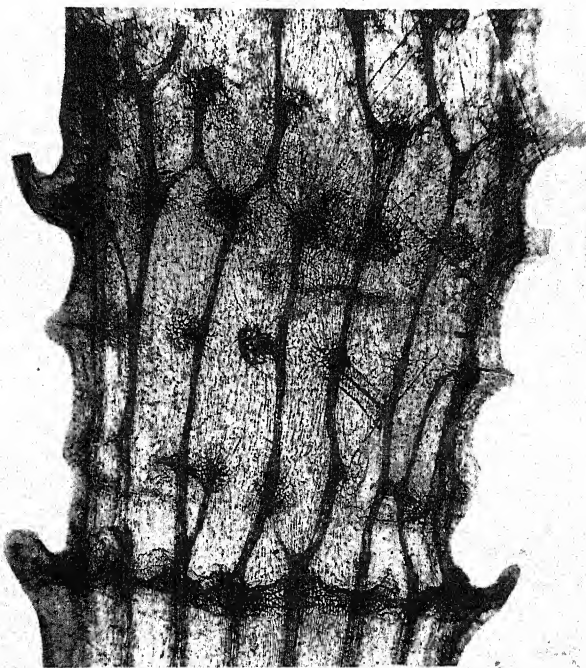
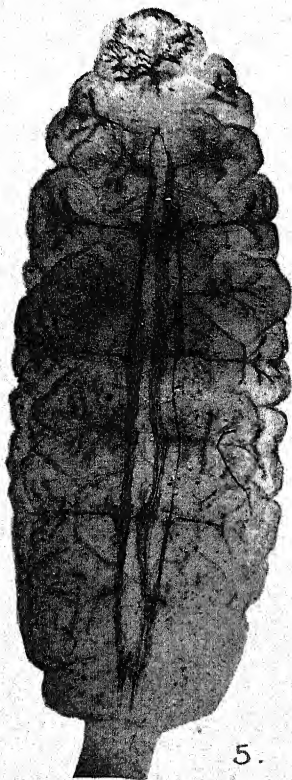
Fig. 6. The vascular arrangement of the cone of *E. limosum*. From a cleared preparation.  $\times 12$ .



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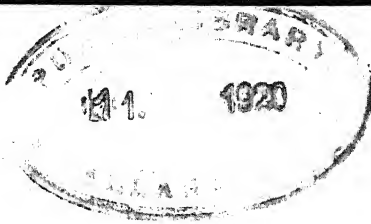






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# A Third Contribution to our Knowledge of the Anatomy of the Cone and Fertile Stem of *Equisetum*.

BY

ISABEL M. P. BROWNE.

With Plates VIII and IX and seven Figures in the Text.

## I. MATERIAL.

THE present investigations were confined to the cone and the region transitional between the latter and the fertile stem in *Equisetum hyemale* (L.) and *E. giganteum* (L.), both species placed by Milde in his genus *Hippochaete* or *Equiseta cryptopora*.

Serial sections were prepared of four cones of *E. hyemale*. Cones A, B, and C were cut into transverse and Cone D into longitudinal sections. Cones A, B, and C were young, and though the vascular system was fully developed, the internodes would presumably have elongated considerably later on. Thus Cones A and C, measured from the insertion of the annulus to the base of the apical prolongation of the cone, were 6.5 mm. and Cone B 6.9 mm. in length. At its widest point the stele of Cone A attained a diameter of 1.5 mm.; that of Cone B one of 1.25 mm., and that of Cone C one of 1.3 mm. Cone D was more fully grown; it had attained the height of 11 mm. and its stele was 1.8 mm. in diameter at its widest point. Presumably Cone A would later have attained to very much the same size as Cone D. In both the lowest whorl consisted of seventeen sporangio-phores. Cones A and D consisted of eleven and ten whorls respectively. Cones B and C were on a slightly smaller scale, the former having nine, the latter eight whorls, while in both the lowest whorl consisted of thirteen sporangiophores.

Duval-Jouve (p. 221) states that the cones of *E. hyemale* are 10-12 mm. long, and consist of 8-10 whorls. These figures agree fairly well with those given above, though two of my cones had eleven whorls. But his further statement that the whorls consist of 7-8 members does not hold good for the four specimens studied by me. The cones now under consideration may have been unusually large. However, in the cone of *E. hyemale* figured by Vaucher, the middle whorls, borne on the widest part

of the cone, seem to have consisted of about twelve sporangiophores (Vaucher, Pl. IX). The middle whorls of Cone A consisted of thirteen, those of Cone B of eleven, and those of Cone C of eleven or ten members. Unfortunately the lower part of the cone is hidden in Vaucher's figure by the uppermost leaf-sheath. It must be remembered that the cone of this species is nearly sessile, and that in nature its lower part remains surrounded by the uppermost leaf-sheath; thus the lowest whorls of the cone, containing the largest number of sporangiophores, are not so readily perceived as the middle whorls. In the middle region, though the axis and axial stele are markedly wider, the sporangiophores are much less numerous.

In Cones C the series of sections extended to the node below the cone. Of *E. giganteum* serial transverse sections were cut of three cones, A, B, and C. The series was continued below Cone A to include the uppermost node of the fertile branch. A fourth cone, D, was cut serially into longitudinal sections, and the node of a fertile stem, E, was cut in transverse serial sections. The following were the dimensions of these cones, all of which appeared to be mature. Height, measured from just below the insertion of the annulus to the base of the apical prolongation of the cone: Cone A 15 mm., Cone B 10.18 mm., Cone C 12.4 mm., and Cone D 14 mm. The diameter of the stele at its widest was a little over 1.5 mm. in Cone A, 1.3 mm. in Cone B, 1.25 mm. in Cone C, and just over 1 mm. in Cone D. Cones A and B consisted of eleven whorls, Cone D of ten, while Cone C consisted of nine or ten, the young sporangiophores at the apex being so crowded and irregularly disposed as to obscure the number of whorls present. In Cones A, B, and D, the highest number of sporangiophores in a single whorl was eleven; in Cone C one of the whorls contained twelve members.

## II. THE SPORANGIOPHORES.

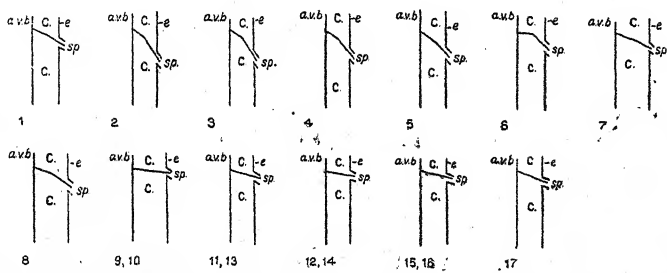
The sporangiophores of *E. giganteum* are short and broad with massive stalks. The traces are the largest I have seen in the genus, and are about two-thirds of the size of the leaf-traces of the fertile branch (Pl. VIII, Fig. 1). Correlated with the shortness of the stalk is the fact that the tracheides in it often begin to diverge fan-wise preparatory to branching before the strand enters the peltate expansion. Indeed, the first branching not uncommonly occurs in the cortex or the trace is prematurely divided and originates as a double strand. The shortness of the stalk of the sporangiophore and the large size of the trace probably account for the numerous cases in which the trace divides prematurely, and is bifascicular at its origin. Among the species studied by me the one that most resembles *E. giganteum* in the size and form of the sporangiophores is *E. palustre*; but in the former these organs are stouter and their traces larger than in the latter species. In *E. giganteum* the massive sporangiophores are



distributed so close to one another round the axis that the tendency to concrescence of sporangiophores, observable in most species of *Equisetum*, is well marked. Often, of course, the concrescence is only partial. I have observed as many as three sporangiophores to be concrescent in their basal portions.

The sporangiophores of *E. hyemale* are less massive and shorter. The transverse sections of my specimens of the cone of this species recall the cones of *E. limosum*. But in *E. hyemale* the sporangiophores and their traces are larger relatively to the axis and the cortex wider than in *E. limosum*. No definite central cavity had made its appearance in my cones of *E. hyemale*, though the central tissues were slightly torn. In specimens of *E. limosum* of the same age and size the development of the central cavity is more advanced.

In *E. giganteum* the traces of the sporangiophores depart either from the middle or from at or near the edge of an axial strand or band of vascular tissue; traces arising in the former position may be termed



TEXT-FIG. 1. Divergence of the sporangiophore-traces of the lowest whorl of Cone A of *E. hyemale*.  $\times 13\frac{1}{2}$ . a. v. b. = axial vascular bundle; c. = cortex; e = epidermis; and sp. = sporangiophore.

median, those in the latter position lateral or slightly internal. Or again, if a bundle remains very narrow when giving off a trace, the latter may be attached to the whole width of the axial strand (cf. Browne (1), pp. 671-2). In *E. hyemale* most of the traces are median or slightly internal, hardly any are truly lateral. In this species, too, the trace may be practically as wide as the strand from which it arises. This is generally so when the strand dies out after giving off a trace.

Though none of my cones of *E. hyemale* were old the sporangiophores and traces of the lowest whorls showed a tendency to be directed obliquely downwards. This phenomenon was most marked in Cone A. Text-fig. 1 reproduces the conditions obtaining in the lowest whorl of this cone. The actual downward deviation is less than in *E. maximum*, for the cone is on a smaller scale; but, as seen in the text-figure, the angle may be rather acute. In this whorl no trace was deflected for more than  $294\mu$ , and the average distance for all the traces of the whorl was but  $124\mu$ . In the whorl

above this one the greatest downward deflexion of any of the traces was  $98\mu$  and the average was  $61.57\mu$ . In the third whorl from the base the oblique course of the traces was hardly noticeable, the average distance in a vertical direction between the point of insertion of the trace on the axial stele and its entry into the sporangiophore being  $28\mu$ . In Cones B and C the average downward deflexion of the traces of the lowest whorl was  $34$  and  $31\mu$  respectively. In the middle region of the cone the traces usually pass horizontally through the cortex, while towards the apex they traverse it in an obliquely upward direction.

### III. THE ANATOMY OF THE CONE.

The tracheides of the cones of *E. hyemale* and *E. giganteum* are essentially similar to those of the other cones studied by me. Those of *E. maximum* are smaller and markedly less strongly lignified, while those of *E. arvense* are very slightly wider and have thinner walls than those of *E. palustre*, *E. limosum*, *E. hyemale*, and *E. giganteum*.

In the last species the radial extent of the xylem in the cone is variable; but on the whole it is greater than in the other species studied. Sometimes the woody tissue is seven or eight cells in depth; in the lower fully developed regions of the cone it is commonly five or six cells in radial thickness (Pl. VIII, Figs. 5 and 8). When the xylem forms narrow bundles this causes the vascular strands to be nearly circular as seen in transverse sections of the axis. Both in *E. giganteum* and in *E. hyemale* unlignified parenchymatous cells, such as occur also in the cones of other species, are found. They are not, however, as numerous as in the cones of *E. maximum*.

In *E. hyemale* the stele is comparatively wide and ovoid as seen in longitudinal section. In *E. giganteum* the cones are longer and relatively more slender. In this species the axis is often slightly enlarged at the insertion of the fertile whorls; this local enlargement frequently affects the diameter of the stele, and in Cone C gives the longitudinal reconstruction a very jagged outline.

In my first paper on the cones of *Equisetum* I pointed out that even a superficial glance at the reconstruction of the stele of a cone which, like Cone A of *E. arvense*, showed a well-developed vascular system, gave an 'impression of nodal bands or a nodal ring of xylem, broken up in the internodes by meshes of parenchyma and usually broken too at the nodes by the persistence of one or two of these meshes' (Browne (1), p. 670). These parenchymatous meshes generally arise vertically above traces that have departed, though at varying distances. As stated on a later page of the same paper (p. 699), the irregular network of strands in the cone of *E. limosum* would seem, when considered separately, to baffle interpretation; but 'considered in the light of a comparative study of the cones of *E. palustre* and *E. arvense*, we see that it has arisen in the phylogeny by

the reduction of xylem, this reduction being manifested by the extension of parenchymatous meshes upwards, downwards, and laterally' (Browne (1), p. 699). In a later paper dealing with *E. maximum* it was pointed out that though the stele of the cone of this species consists of a very irregular network of strands, these irregularities were chiefly due to the poor development of axial xylem at the nodes, so that parenchymatous meshes arising above the traces of a whorl persisted through more than one internode, sometimes through numerous internodes. In Cone B of *E. maximum* one of the meshes extended into seventeen internodes. In this species the poor development of the axial xylem often caused the meshes of the second or higher orders to be widened, either suddenly or gradually, above the nodes by the dying out of the tracheides above a lateral or slightly internal trace. A tendency was further noted in this species for a parenchymatous mesh to become decurrent for a little distance below and to one side of the trace above which, speaking phylogenetically, it may be considered to have arisen (Browne (2), p. 235).

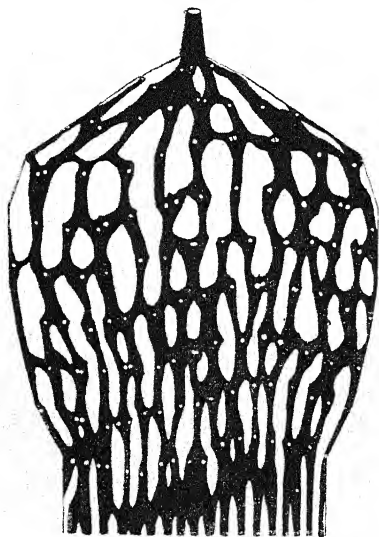
A study of the reconstructions of the steles of the cones of *E. hyemale* (Text-figs. 2, 3, and 4) and *E. giganteum* (Text-figs. 5, 6, and 7) will show that here too a large number of meshes are closed at or near the level of a fertile node, and that fresh meshes usually make their appearance at a little distance vertically above traces that have departed. Usually the amount of xylem present at the nodes is insufficient to close all the meshes of the internode below (cf. Pl. VIII, Fig. 8). Consequently some persist through each node, except through the seventh node of Cone A of *E. giganteum*. Here all the meshes are closed, though no single section shows a complete ring of xylem, because all the traces are not inserted at precisely the same level, and one of the meshes is only closed after some of the meshes of the internode above have made their appearance.

Although none of my reconstructions of the cones of *E. hyemale* show closure of all the meshes at any one node (except at the extreme apex of Cone B), the origin of the great majority of the meshes vertically above traces is perhaps more marked in the well-developed stele of Cone A of this species than in the steles of any other cones studied by me, except those of *E. arvense*. Moreover, Cone A shows an unusually high proportion of meshes of the first order. Cones B and C of this species have a less developed vascular system.

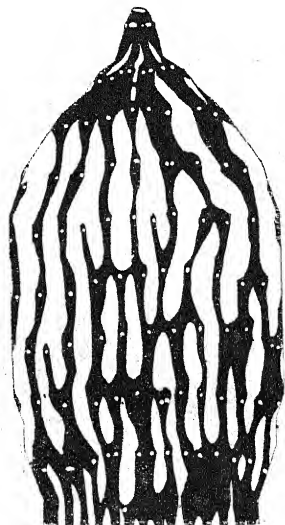
Forty-eight meshes arise and are closed within Cone A of *E. hyemale*. Two more unclosed meshes arise above traces of the eighth whorl and become confluent round the vascular strand of the terminal acumen. Of the forty-eight meshes I regard twenty-seven as of the first, sixteen as of the second, three as of the third, and two as of the fourth orders. Nine more meshes arise above the annulus; two of these are closed at or near the insertion of the lowest whorl of sporangiophores, and may be regarded as of

the first order (cf. pp. 252-3). The remaining seven persist into the cone, five being of the second and two of the third orders. Of the eighteen meshes arising above the last whorl of leaves thirteen are of the first order, being closed in the neighbourhood of the insertion of the annulus. Of the remaining five three are of the second order and are closed near the level of the lowest whorl of the cone, while two, both of the fourth order, persist into the cone.

In Cone B of the same species twenty-two meshes arise and are closed : six of the first, four of the second, three of the third, three of the fourth, two of the fifth, and two of the sixth orders. Five meshes originate above



TEXT-FIG. 2. Longitudinal reconstruction of the xylem of Cone A of *E. hyemale*. The xylem of the stele is drawn as though cut and spread out flat. Axial xylem black, traces and parenchyma white. Magnification *circa* 10.



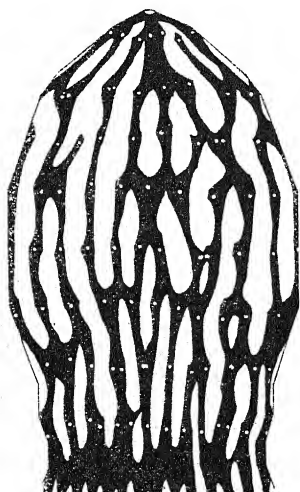
TEXT-FIG. 3. Longitudinal reconstruction of Cone B of *E. hyemale*. Axial xylem black, traces and parenchyma white. Magnification *circa* 10.

the annulus ; one only is of the first order and is closed just above and between the insertion of two of the sporangiophores of the basal whorl of the cone. Four persist into the latter, three being of the second and one of the seventh orders. Of the fourteen meshes arising above the uppermost whorl of leaves nine are closed near the level of insertion of the annulus and are, therefore, of the first order. Four of the second order are closed at the level of the lowest fertile node, and one, of the fourth order, extends upwards into the cone.

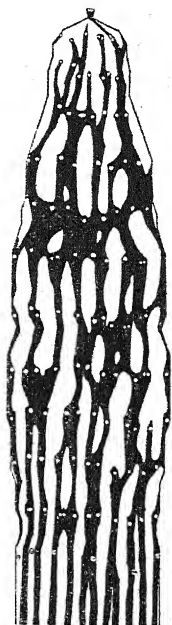
In Cone C of *E. hyemale* twenty meshes arise and are closed : five of the first, eight of the second, two of the third, one of the fourth, and four of the fifth orders. In this cone eight meshes arise above the level of the annulus ; one, of the first order, is closed just above the level of the lowest

whorl of the cone; seven persist into the cone—three of the second, two of the third, one of the fifth, and one of the ninth orders. Above the last whorl of leaves fourteen meshes are initiated; twelve of these are of the first order and are closed in the neighbourhood of the insertion of the annulus, and two of the third order extend into the cone.

Turning to *E. giganteum*, twenty meshes originate and are closed in Cone A of this species: thirteen of the first, five of the second, and two of



TEXT-FIG. 4. Longitudinal reconstruction of the xylem of Cone C of *E. hyemale*. Axial xylem black, traces and parenchyma white. Magnification *circa* 10.

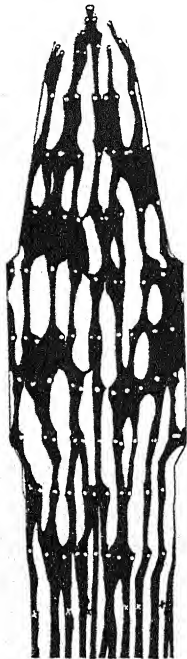


TEXT-FIG. 5. Longitudinal reconstruction of the xylem of Cone A of *E. giganteum*. Axial xylem black, traces and parenchyma white. The circles, black on white and white on black, with a bar across them, represent strands passing from the annulus into the axis, but dying out before reaching the stele on the radii of the strands or meshes on which they are shown. Magnification *circa* 5.

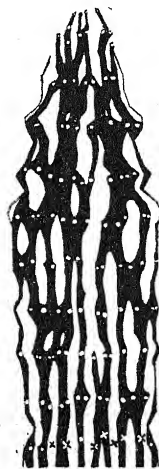
the third orders. Six more unclosed meshes originate in the upper part of the cone. No fresh meshes arise between the insertion of the annulus and the lowest whorl of sporangiophores in any of the cones of this species examined by me. But from analogy with other species, as well as from the fact that a certain number of meshes are constantly closed in the region of insertion of the annulus, the latter may presumably be regarded as a reduced whorl, and held to mark the position of a node when estimating the order of the meshes that originate above the last whorl of leaves (cf. also pp. 257-8). In Cone A there are eleven of these: two of the first order and

two of the second order are closed respectively at the level of the annulus and of the basal whorl of sporangiophores; of the seven that enter the cone two are of the third, one of the fourth, two of the fifth, and two of the seventh orders.

In Cone B of this species twenty-three meshes originate and are closed: thirteen of the first, four of the second, three of the third, two of



TEXT-FIG. 6. Longitudinal reconstruction of the xylem of Cone B of *E. giganteum*. Axial xylem black, traces and parenchyma white. The crosses, black on white, and white on black, represent strands passing from the annulus into the axis, but dying out before reaching the stele on the radii of the strands or meshes on which they are shown. Magnification circa  $7\frac{1}{2}$ .



TEXT-FIG. 7. Longitudinal reconstruction of the xylem of Cone C of *E. giganteum*. Axial xylem black, parenchyma and traces white. The crosses, black on white, and white on black, represent strands entering the axis from the annulus, but dying out before reaching the stele on the radii of the strands or meshes on which they are shown. Magnification circa 5.

the fourth, and one of the fifth orders. Three more meshes originate in the upper part of the cone and remain unclosed. Of the eleven meshes found in the branch above the last whorl of leaves two, of the first order, are closed below the insertion of the annulus, and three, regarded as of the second order, are closed at or near the level of the lowest sporangiophores. Six of these meshes therefore enter the cone: three of the third, one of the fourth, and two of the fifth orders.

In Cone C of *E. giganteum* eighteen meshes originate and are closed: ten of the first, six of the second, one of the fourth, and one of the sixth



orders. Four unclosed meshes arise in the upper part of the cone. Above the last whorl of leaves the axis contains ten parenchymatous meshes. Three of the first order are closed in the neighbourhood of the insertion of the annulus on the axis. (The relatively wide third strand of the reconstruction arises by the fusion of two strands leading to the closure of a mesh below the level of the diagram.) Of the remaining seven meshes one is of the second order, being closed below the departure of the traces of the lowest whorl of sporangiophores; six extend into the cone—one of the third one of the fourth, two of the fifth, one of the sixth, and one of the eighth orders.

The widening of parenchymatous meshes commonly occurs by the dying out of tracheides above lateral or slightly internal traces, so that a mesh may become laterally biseriate in the second or later internodes through which it pursues its course. In Cone A of *E. hyemale*, however, the mesh lying above the eleventh and twelfth traces of the fifth whorl is laterally biseriate at its origin, i.e. the meshes above two sporangiophores are congenitally fused. The mesh subtended by the bifascicular trace of the third sporangiophore of the sixth whorl of the same cone, though of much the same shape, is not truly laterally biseriate, as both bundles of this prematurely divided trace enter a single sporangiophore (cf. Text-Fig. 2).

There is one consideration in connexion with the closure of parenchymatous meshes the significance of which was not as much emphasized as it should have been in my earlier papers on the cone of *Equisetum*, though the point was touched upon (Browne (1), pp. 680-1). It has already been pointed out that when the reduction in the amount of xylem at the nodes of the cone is greater than the reduction in the diameter of the stele, this leads to the formation of fewer meshes; but of meshes of a higher order (Browne (1), pp. 672-3, and (2), pp. 235 and 259). It is obvious that where a mesh is much narrowed at the height of a node and widens out again in the internode above, the formation of but little more axial xylem at the level of insertion of the sporangiophores would convert a mesh of a higher order into two of lower orders. For example, the presence of but a small amount of additional axial xylem between the sixth and seventh traces of the fourth whorl of Cone B of *E. giganteum* or between the seventh and eighth traces of the fifth whorl of Cone A of *E. hyemale* would, in the first case, convert a mesh of the fifth order into two meshes, the lower of the third and the upper of the second orders. In the second case it would substitute for a mesh of the third order two meshes, the lower of the first and the upper of the second orders. Similarly, the production of a little more axial xylem between the fifth and sixth traces of the second whorl of Cone A of *E. arvense* (Browne (1), p. 667), between the fourth and fifth traces of the fourth whorl of Cone A of *E. palustre* (Browne (1), p. 671), between the thirteenth and fourteenth traces of the second whorl of Cone B

of *E. limosum* (Browne (1), p. 677), and between the thirteenth and fourteenth traces of the fourth whorl of Cone B of *E. maximum* (Browne (2), Pl. XIII) would convert single meshes, narrowed at a node, into two meshes of lower orders. The cases quoted are merely examples and do not constitute a list of the cases in which this phenomenon can be observed, and the reader who studies my reconstructions of the steles of cones will be able to find plenty of other examples. I have already pointed out that, owing to this correlation between the closure and origin of meshes and the amount of axial xylem at a node, the proportion that the number of meshes bears to the number of sporangiophores affords a general indication of the comparative reduction or good development of the vascular tissues of the cone. The smaller the proportion of meshes compared with the number of sporangiophores the greater the reduction of the xylem (Browne (2), p. 236). This, however, is only true without further modification of the cones of *E. arvense*, *E. hyemale*, and *E. palustre*, in which the meshes are closed, generally speaking, by the formation of additional tracheides at the nodes. In *E. giganteum*, *E. maximum*, and *E. limosum*, some of the meshes are closed in this way; but, commonly in *E. maximum* and in *E. limosum* and more rarely in *E. giganteum*, meshes are closed also by the obliquely divergent course of some of the tracheides just above the node. When the nodal xylem is relatively well developed a trace-bearing strand, though not wide enough to unite with neighbouring strands, is still wide enough to give off a median trace, above which a fresh mesh arises. In such cases the closure of the mesh of the internode below is often affected by the obliquely upward course of one of the branches (into which the appearance of the new mesh has divided the trace-bearing strand) and its fusion with the adjacent strand or branch of a strand. If both branches of a trace-bearing strand fuse with neighbouring bundles the meshes lying on either side of the strand in the internode below are closed. When the nodal xylem is even more reduced and a trace departs from an isolated strand, too narrow for the formation of a fresh mesh above such a trace, the unbranched strand may close one of the meshes of the internode below by its oblique course and ultimate fusion with a neighbouring bundle. The closure of a mesh by the fusion of two such narrow, unbranched strands is especially characteristic of nodes above which reduction in the number of members in a whorl and of internodal strands is about to occur, for example of the apices of cones. Not uncommonly, in all cones, a mesh is closed partly by the formation of additional tracheides at the edge of one of the strands bordering on a mesh, and partly by the oblique course of the tracheides of the bundle, or branch of a bundle, on its other side. Where the closure of a mesh is due to the oblique course of the tracheides above a node the closure is naturally only effected above—sometimes slightly, sometimes considerably above—the node. Where the fusion of the strands occurs but little above the node,



I have not considered the mesh thus closed as of a higher order than those closed at or just below the node. There seems little reason to doubt that this mode of closure of a parenchymatous mesh is secondary and due to poor development of xylem at the node. The amount of xylem at this level being insufficient to close the meshes this closure is effected slightly higher up by the oblique course and fusion of adjacent strands or their branches.

It is not unlikely that, as occasionally in cones of *E. giganteum*, so also in those of *E. arvense*, *E. hyemale*, and *E. palustre*, the closure of meshes in cones with relatively wide steles and little axial xylem may sometimes be effected by the oblique course of the tracheides, especially where the origin of a mesh causes the branching of an isolated trace-bearing strand; but this form of closure is not characteristic of these species. As such a mode of closure of meshes does not imply an increase in the amount of xylem locally present, the cones of *E. maximum* and *E. limosum*, in which it is of widespread occurrence, have relatively even more reduced vascular systems compared with *E. arvense* than is indicated in the table of statistics on p. 236 of my second paper on *Equisetum* (Browne (2)). Naturally, too, the tracheides running obliquely—some of them transversely—across a mesh occupy a wider space in the longitudinal reconstruction than would an equal number of tracheides, the long axes of which were vertically directed.

Relatively to the number of sporangiophores there are many fewer parenchymatous meshes in the cones of *E. hyemale* and *E. giganteum* than in those of *E. arvense*. If the small number of cases examined is representative, the average proportion of meshes to sporangiophores is, in *E. arvense*, rather more than twice as great as in *E. hyemale*, and rather more than three times as great as in *E. giganteum*. Indeed, judged by this standard alone, the cones of the last-mentioned species would have the most reduced vascular systems of any, the anatomy of which is known to us, the proportion of meshes to sporangiophores being very slightly lower than in the cones of *E. maximum* and *E. limosum*. Such a conclusion would, however, be erroneous. In most of its characters the cone of *E. giganteum* shows that it possesses a relatively more developed vascular system than the cones of *E. palustre*, *E. maximum*, and *E. limosum*. For example, the proportion of meshes closed by additional development of tracheides at the node rather than by their oblique course is very much greater in *E. giganteum* than in *E. maximum* or *E. limosum*. Then again, the proportion borne by the meshes of the first and second orders to the total number of meshes, though rather lower in *E. giganteum* than in *E. arvense*, is much higher than in *E. palustre*, *E. maximum*, or *E. limosum*. In two respects the cone of *E. giganteum* affords an example of relatively greater development of the xylem than even that of *E. arvense*. Firstly, as already mentioned (p. 240), the xylem tends to attain a greater radial depth than in

the other cones with the anatomy of which we are acquainted. No great radial depth of xylem is found, but the tendency is well marked and should be borne in mind in considering the longitudinal reconstructions of the axial stele, in which it is, of course, not apparent. Secondly, continuous tracts of xylem extending uninterruptedly above some of the traces of a whorl to other traces of the whorl above were a more marked characteristic of the cones of *E. giganteum* than of those of any other species yet examined by me. These sweeps of internodal xylem form very obvious features in the longitudinal reconstructions of the steles of Cones A and B, and occur also in that of Cone C, the xylem of which is less well developed.

Such continuous internodal tracts of xylem seem to be uncommon in the cones of *Equisetum*; but I have found them in all cones of *E. arvense* of which I possess serial sections of any considerable part of the cone. They occur, too, in the cones of *E. hyemale*, *E. palustre*, and *E. maximum*. In the two former species they are found chiefly above the annulus and in the upper, narrower part of the stele. In the latter position I am inclined, as already stated (Browne (2), p. 260), to regard their presence as resulting from the considerable reduction in width of the stele. Such an explanation does not account for their occurrence in the cones of *E. arvense* and *E. giganteum*. Here their presence, which involves the absence of a mesh over one or more median traces, is an indication of considerable development of xylem. Incidentally I may state that I have not observed in *E. limosum* any case in which considerable bands of xylem extended through a whole internode. Occasionally, however, two bundles that have fused and have given off two lateral traces, one from each end of the woody band, remain united into a wide strand in the internode above; e.g. the strand from which the third and fourth traces of the fourth whorl of Cone A of *E. limosum* have departed (Browne (1), p. 676). We cannot here, however, speak of the absence of a parenchymatous mesh, since such meshes do not arise above lateral traces. Not only are these comparatively wide sweeps of internodal wood common in *E. giganteum*, but I have observed that they may involve the absence of a mesh over as many as three laterally consecutive, median traces, that is over a larger number of traces than in the other species studied.

It will be remembered that in the cone of *E. maximum* a tendency resulting in increase of axial xylem was noted: strands were often linked up by the formation of additional xylem below the departure of traces (Browne (2), p. 237). This character is met with also, though more rarely, in the cones of *E. palustre* (cf. the reconstruction of the stele of Cones A of this species, Browne (1), p. 671), and is found fairly often in the cones of *E. giganteum*. When it is found at a level at which the internodal sweeps of xylem referred to above are present, there arise wide internodal bands of xylem separated by two or three small, free bundles, or in extreme cases

the internodal stele assumes the appearance of a ring broken only in two places by a single, small, separate strand (Pl. VIII, Fig. 6). In *E. hyemale* it is rare for the strands to become united much below the level of the fertile node.

Taking into consideration the various factors that contribute to the development of the vascular system in the cones of *Equisetum* it is clear that the species in which the xylem of the cone is relatively best developed is *E. arvense*. The cones in which the axial xylem is relatively most reduced are those of *E. maximum* and *E. limosum*—the xylem of the cone of the latter being, it would seem, comparatively slightly more reduced than that of the cone of the former. The cones of *E. hyemale*, *E. palustre*, and *E. giganteum* represent the middle term of such a series. The cones mentioned do not, of course, constitute a phylogenetic series. On the whole the vascular system of the cone of *E. palustre* is rather less well developed than that of *E. hyemale*. A comparative estimate of the reduction of the stele of the cone in *E. giganteum* on the one hand, and in *E. hyemale* and *E. palustre* on the other—or indeed of the vascular system of the cone of *E. giganteum* and that of other species of the genus—is very difficult to carry through. This is because in the cones of *E. giganteum* two opposite tendencies are at work: firstly, a tendency for the amount of xylem developed to be comparatively large, owing chiefly to the formation of wide internodal sweeps of xylem, but also partly to the closure of parenchymatous meshes some distance below the node and to the radial extent of the xylem; secondly, a tendency for relatively little xylem to be developed owing to the persistence of some parenchymatous meshes through a considerable number of internodes. Neither tendency is peculiar to *E. giganteum*, though in no other among the species studied is the formation of internodal sweeps of xylem so marked a character. It is the combination of these two tendencies that makes it difficult to estimate the position of this species in a series showing gradual reduction of the xylem. Perhaps its cone may be best regarded as having relatively more xylem than that of *E. palustre*, but comparatively a little less than that of *E. hyemale*.

#### IV. ALTERNATION AND SUPERPOSITION OF THE TRACES.

At the periphery of the axis of the cones of *E. hyemale* and *E. giganteum*, the sporangiophores of successive whorls alternate regularly with one another, except where there is a change in the number of members. Even in the latter case the area of disturbance of alternation is generally restricted. Usually the young sporangiophores are closely imbricated, their peltate expansions being accurately fitted in with those of the whorls above and below. No such regular alternation of the traces prevails at their insertion on the stele. The persistence of parenchymatous meshes through more than two internodes, of course, makes it impossible for the traces of a whorl

to be accurately superposed to those of the second whorl in a downward direction, as they would be if the traces of successive whorls alternated quite regularly. Speaking generally, where sufficient nodal xylem is developed for a number of laterally consecutive meshes to be closed, the traces given off from the band that is produced by these closures alternate more or less regularly with those of the whorl below. More or less regular superposition occurs, as in the other species studied, when the meshes on either side of a strand persist unnarrowed through a node, so that the strand gives off a trace without afterwards branching, i.e. without the formation of a mesh above the trace. Superposition of traces is not so common in the cones of the two species under consideration as in those of *E. maximum* and *E. limosum*, because the axial xylem is relatively better developed. Examples, however, occur in the three cones of *E. hyemale* as well as in the three of *E. giganteum* of which longitudinal reconstructions of the axial stele were made (cf. Text-figs. 2, 3, 4, 5, 6, and 7). In the last species I have not observed more than three successive traces to be inserted in the same vertical line, whereas in *E. maximum* and *E. limosum* the number may be as high as seven. In Cone A of *E. hyemale*, a cone which had a considerably better developed vascular system than Cones B and C of the same species, accurate superposition of the traces was rare, and I did not observe more than two successive traces to be inserted on the same vertical line. In Cones B and C of this species, however, I found that the number might be as high as four to six. Irregular alternation of traces is common in both species (cf. Browne (2), p. 239), and occurs most often when a band of xylem gives off one or more median traces and a lateral trace at either or each end. When we are not dealing with cases in which wide sweeps of xylem persist through the internode above, a parenchymatous mesh (or meshes) arises over the median trace (or traces) of such a band. No fresh mesh, of course, arises over the lateral trace or traces, and the strands formed by the breaking up of the band alternate irregularly with those in the internode below. When the strands narrow markedly above the node, owing to the dying out of the tracheides immediately above the lateral trace, the irregularity in the alternation is less.

Both in *E. giganteum* and in *E. hyemale* the nodes of the cone vary very greatly in the amount of axial xylem developed, and therefore in the number of meshes closed. In *E. giganteum* it is common for some trace-bearing strands to remain isolated, and for others to become united in pairs, each pair remaining separated from neighbouring strands by relatively wide parenchymatous meshes. In such cases the incoming traces, which are, of course, entering the cortex from sporangiophores arranged regularly around the axis without regard to the persistence of wide parenchymatous meshes through the node, naturally tend to be inserted at or near each end of the band of xylem formed by the fused strands. Above such traces fresh

parenchymatous meshes cannot very well arise, and the prevalence of these bands, dual in origin and retaining their dual character, is no doubt one of the reasons why the cone of this species has so few parenchymatous meshes in spite of a relatively considerable amount of axial xylem. In the internode above these pairs of strands usually remain fused and form a complex or double strand which, though narrowing slightly, remains markedly wider than the single strands of the internode. At the next node these double strands may once more widen and again give off two lateral—or nearly lateral—traces more or less superposed—sometimes accurately so—to those of the whorl below (e.g. the sixth strand between the first and second whorls of Cone A of *E. giganteum*). More often, however, a double strand unites at the next node with another double or a single strand or strands, and the resulting complex or band of xylem gives rise to a group of traces corresponding in number to its constituent parts and alternating irregularly with those of the whorl below (e.g., the fifth, sixth, seventh, and first strands of the internode between the third and fourth whorls of Cone B of *E. giganteum*).

As in the other cones studied by me, so in those of the two species now described, the parenchymatous meshes arise in by far the greater number of cases, vertically above traces that have departed. I have not observed in *E. hyemale* any examples of the tendency, so characteristic of the cone of *E. maximum* for parenchymatous meshes to become decurrent below and to one side of the traces that may be held, speaking phylogenetically, to subtend them. Such behaviour of the meshes is rare in *E. giganteum*, but an example may be observed in the mesh above the eighth trace of the fifth whorl of Cone A.

On the other hand, in *E. giganteum* more often than in the other species studied by me, meshes do arise not superposed to traces. In other words, strands branch and the point of branching does not lie vertically above a trace; moreover, this branching is not necessarily associated with an increase in the number of traces in the whorl above. Still, even in *E. giganteum* this origin of a parenchymatous mesh between rather than vertically above traces is rare; and this though the prevalence in this species of 'double strands' giving off two lateral traces would seem to invite branching of the strand above and between the traces. In the great majority of cases these bands of xylem, dual in nature, that have given off two lateral or slightly internal traces, persist undivided through the internode above.

#### V. APEX OF THE CONE.

Both in *E. hyemale* and in *E. giganteum* the cone normally ends in a pointed apical prolongation, Duval-Jouve's acumen. In *E. hyemale* this is traversed by a vascular strand which is much stouter than an ordinary

trace, and is formed by the prolongation and condensation of the product of fusion of all or several of the strands of the cone. In *E. giganteum* the acumen is usually penetrated by a single, very narrow strand which widens out slightly higher up. In Cone C two very narrow strands, the middle ones of the longitudinal reconstruction, pass into the acumen. In this species the other strands of the cone pass out into sporangiophores of the uppermost whorl.

At its base the acumen rests upon a rounded, lobed, parenchymatous cushion, apparently representing the concrescent primordia of incompletely developed sporangiophores. These lobes (or sporangiophores) are adnate by their upper surfaces to the acumen. In *E. giganteum* some of the lobes commonly bear on their lower surface small sporangia, closely fitted into and dovetailing with those borne on the upper side of the obliquely upwardly directed sporangiophores of the highest whorl. The sporangiophores of this whorl are short-stalked structures, partially concrescent with one another, and intermediate in appearance between the other sporangiophores and the adnate lobes. In *E. hyemale* as a rule the sporangia of the uppermost whorl of sporangiophores are closely fitted into the parenchymatous cushion at the base of the acumen, and the lobes of the cushion usually bear no sporangia. But in Cone D one of the sporangiophores of the highest whorl was inserted rather lower down than the others, and above it a small sporangium was produced on the lower surface of one of the lobes adnate to the base of the acumen.

## VI. THE REGION TRANSITIONAL FROM FERTILE STEM TO CONE.

### A. Description in *E. hyemale*.

As we reach the level of the insertion of the annulus in *E. hyemale* most of the strands of the internode above the uppermost whorl of leaves become united. Slightly higher up numerous fresh meshes arise. In none of the species that I have studied are the fusion of the strands at or below, and their branching above, the insertion of the annulus so marked a feature as in *E. hyemale*. Relatively few meshes persist through this level, and the nodal appearance of the region is very striking in all the longitudinal reconstructions of the axial stele (cf. Text-figs. 2, 3, and 4). In all cases more meshes are closed at or near the insertion of the annulus than arise above it. Comparatively wide sweeps of xylem extending through the whole of the internode between the annulus and the basal whorl of sporangiophores are characteristic of this species. Altogether the development of xylem in this region is relatively greater than in any other species the anatomy of which is known to us. I think, however, that the fact that a comparatively large number of meshes are closed in the neighbourhood of the annulus in this species is partly due to the narrowness of the stele at the

base of the cone and to the relatively large number of the strands here, and of members in the lowest whorl of sporangiophores. Where the stele is widest—in the middle or upper part of the cone—much more interfascicular xylem would be necessary to close an equal number of meshes. Here the internodal strands are far less numerous, and the diameter of the stele much greater, so that the internodal bundles are widely separated (cf. Pl. IX, Figs. 11 and 12).

In Cones A and B of this species a single trace was given off at or near the level of the annulus. Unfortunately, its outward course could not be followed, as just in this region the cortical tissues and those external to them had been injured by the hydrofluoric acid used to desilicify the cones. It was not even clear whether this trace ran to the base of a sporangium or not. In Cones C and D there was no sign of any such abnormality. It may be added that Milde especially states that the annulus at the base of the cone is quite normal in structure (Milde, p. 514), and that Duval-Jouve includes it among the species that do not show 'irregularities of the annulus' (Duval-Jouve, p. 154).

#### B. Description in *E. giganteum*.

In the internode of the fertile branch of *E. giganteum* the central cavity is very large. In both my specimens, in the axis below Cone A and in branch E, the internodal bundles are eleven in number, each surrounded by a separate endodermis. The vallecular canals are conspicuous and the carinal ones show a tendency to be wider transversely than radially (Pl. VIII, Figs. 3, 4, 7, and 9). The bundle in this region is approximately circular as seen in transverse section of the stem. The two lateral bands of metaxylem usually run straight outwards, nearly parallel to one another, and are inserted more or less at right angles to the middle group of xylem, usually chiefly represented by the carinal canal. The number of tracheides in the lateral bands seems to vary from five to twelve, but it is usually greater than in the internodal bundle of the stem figured by Milde, where there are only five tracheides (Milde, Pl. XXI, Fig. 4). Not infrequently the bands of metaxylem converge towards the periphery of the stele. As we approach the node the central cavity narrows, but there is no persistent diaphragm at this level. As the internodal bundles become laterally united into a ring the separate endoderms are replaced by a common inner and outer endodermis. The structure of the node is essentially that characteristic of the genus. Above the last vegetative node the central cavity widens somewhat, though it does not attain to the same width as in the internode below. The separate bundles, each with an endodermis, are reconstituted and alternate with those of the previous internode. The carinal cavities reappear, but the vallecular canals are not re-formed. Milde asserts that the branches have six to eight, very rarely nine, ribs and bundles (Milde, p. 399). As



already stated, both my specimens had eleven bundles ; moreover, from the structure at the base of Cones B and D it is clear that the latter cones were borne on axes with eleven bundles. In Cones A and B, however, one of the bundles was very small and poorly lignified. In Cone C there are ten internodal strands presumably arising above and between an equal number of leaves.

The U-shaped or horseshoe-like distribution of the tracheides in the bundles does not always persist for any considerable distance. Sometimes the lateral arms flatten out rapidly as we pass upwards. The more internally situated elements may come to lie in the continuation of the curve, slightly concave outwards, formed by the carinal group of tracheides, while the peripheral elements may cease to develop as tracheides. In other cases there is, for a short distance, no lateral metaxylem, so that, most of the protoxylem having disappeared, the mature stem may possess very little xylem at this level. If the U-shaped distribution of the xylem is temporarily lost, it is restored below the insertion of the annulus ; in other cases the xylem of each bundle remains more or less U-shaped, until just below the point at which the annulus becomes free from the axis. At this level the xylem assumes the compact form characteristic of the bundles of the cone. Between the annulus and the last whorl of leaves the bundles not infrequently contain one to four, commonly two or three, rather wide tracheides adherent to the inner wall of the carinal canal. In other words, the first elements to be destroyed by the formation of the carinal canal are not the most internally situated. Developmental stages were not available, but it seems possible that these elements may have been centripetal xylem (cf. Pl. VIII, Figs. 3, 4, 7, and 9). Below the annulus the bundles not infrequently contain one or more laterally situated tracheides much larger than the others.

The annulus is adherent to the stem by its wide base, giving the latter a funnel-shaped outline. The free edge is slightly incurved and appears to be normally sporangiferous (Pl. VIII, Fig. 2). The sporangia, which are about half the size of those borne by the sporangiophores, are completely hidden by the incurved edge of the annulus, and no trace of them was observable until the cones had been sectioned. In all the specimens examined the annulus contained a certain number of vascular bundles running to the insertion of the sporangia. These are attached by their upper ends to the incurved edge of the annulus. Not all of the vascular bundles are connected with axial strands ; as a rule the greater number, sometimes all of them, die out without reaching the axial stele. Such bundles may conveniently be described as free (annular) bundles. They are shown in the reconstructions, black on white and white on black, on the bundles and parenchymatous meshes on the radii of which they die out. In the reconstruction of Cone A they are distinguished from the traces

given off from the axial bundles by a horizontal line across them, while in those of Cones B and C they are shown as crosses. The annular traces proper, i. e. the strands that are in connexion with the axial bundles, at first pursue a steeply upward course. In a transverse section of the stem at the point at which the annular traces become free, the tracheides of the latter are cut almost transversely, and offer a striking contrast to those of the traces departing to sporangiophores; for the traces of the latter pass out approximately horizontally and their traces are, therefore, cut more or less longitudinally in a transverse section of the axis. Soon, however, the annular trace bends sharply out into the parenchymatous tissue of the annulus; in the region of the bend there is usually a slight downward bowing of the annular traces, but they soon run outward and upwards. The traces are, usually at least, somewhat smaller than those of the sporangiophores of the whorl above them. Some of them fork near the free edge of the annulus, and the branches or unbranched bundles run to the point of insertion of the sporangia. The sporangia borne by the annulus are fitted in very closely with those of the downwardly directed sides of the lowest sporangiophores, and transverse sections of the axis in this region frequently pass through sporangia borne by the sporangiophores as well as those borne on the annulus.

The number, distribution, and behaviour of the annular bundles vary considerably in the different cones. The vascularization is most pronounced in Cone C, where the number of annular bundles is eleven, only one less than the number of the traces and sporangiophores in the lowest whorl of the cone. Four of these bundles are in connexion with the axial strands, and are hardly smaller than the traces of the sporangiophores immediately above. Each of these four annular traces forks once, and one of the branches forks again. Each of the nine strands thus formed runs to the insertion of a sporangium. The remaining seven strands are free annular bundles. There is no sharp line of differentiation between the tissues of the axis and those of the annulus. As the latter is adherent by its wide base to the stem, the stele, just below the level at which the annulus detaches itself, is surrounded by a wide parenchymatous cylinder in which the free annular bundles end blindly at very different depths. Four of them are unbranched and terminate at the insertion of a sporangium; the three others fork near the edge of the annulus and each branch runs to the point of insertion of a sporangium. In this cone the annulus bore nineteen sporangia.

The annulus of Cone A contains six vascular bundles, none of which enter into complete connexion with the axial bundles. The third, as seen in the reconstruction of the stele (Text-fig. 5), approaches very near to the central cylinder, and one or two phloem-elements appear to pass out from the axial bundle, but no connexion is established between the tracheides of

the two strands. Five of the annular bundles run unbranched to the points of insertion of an equal number of sporangia, while the sixth (the third of the reconstruction) forks and each branch terminates at the point of insertion of a sporangium. The annulus below Cone A only bore six sporangia.

Cone D showed six annular bundles; two unbranched, each ending at the point of insertion of a sporangium, were connected with the axial bundles. Of the four free bundles three forked in the upper part of the annulus, and each of the seven strands thus formed ran to the point of insertion of a sporangium, of which in this cone the annulus bore nine.

The sections of the annular region of Cone B were injured in mounting, and the details of the annular bundles and their relation to those of the axis could not be satisfactorily followed. There were at least six and probably not more than eight annular vascular strands, most of them apparently free bundles. In the reconstruction of the stele of this cone (Text-fig. 6), the number and position of the annular strands must only be considered to be approximately indicated.

In 1867 Milde described and figured the cone of *E. xylochaetum*, Mett., a species allied to *E. giganteum*. After stating that the cone consists of eleven whorls he proceeds: 'The lowest whorl, an annulus of very definite form (Fig. 29), is always cup-shaped with eight erect lobes; its component parts are not separate, but fused with one another as occurs in the sheath.<sup>1</sup> Each lobe bears on its inner surface and at its base a single sporangium' (Milde, p. 383). Unfortunately this author neither describes nor figures the cone of *E. giganteum*. The sporangiferous annulus naturally strengthens the relationship of the two species; but if my specimens and Milde's are typical the species show numerous differences of detail. My cones were half as large again as his; the annulus seemed to consist of rather more (nine to eleven) lobes and the sporangia were more variable in number, being sometimes, at least, much more numerous. These differences may be partly due to the greater size of the cones of *E. giganteum*: but the difference in the attachment of the sporangia seems to be of more importance. Milde speaks of the sporangia as being inserted *singly* at the *base* of the lobes of the annulus, while in *E. giganteum* they are inserted on the incurved upper edge of the annulus, and do not necessarily stand in any definite numerical relation to its lobes.

## VII. DISCUSSION CONCERNING THE PRIMITIVE FORM OF THE ANNULUS.

Though in *E. giganteum* the annulus contains a certain number of vascular strands extending inwards from the sporangia towards the axial stele, and though some of these strands may become connected with the axial bundles, yet it is just this species in which the node-like character of

<sup>1</sup> i. e. leaf-sheath.

the axis at the point of insertion of the annulus does not show itself in the formation of fresh parenchymatous meshes above this region. I am inclined to think that the development of normally sporangiferous annuli is a new character in the phylogeny of the genus, though sporangiferous annuli are not uncommon as abnormalities in several species. In other words, I believe that the sporangia have, in the phylogeny, spread to regions which were not at first sporangiferous. If the development of sporangia on the Equisetaceous annulus is a fresh character it would necessitate the provision of a vascular supply for the nutrition of the spores. The fact that all the vascular strands observed in annuli of *E. giganteum* ran to points of insertion of sporangia, and that the point of insertion of each sporangium was marked by the termination of a vascular strand, would seem to show that there is a correlation between the development of sporangia and vascular strands by the annulus. In fact, if the development of sporangia on the annulus be regarded as a character acquired relatively recently in the phylogeny, it is hard to escape from the conclusion that the vascularization of the annulus is a change connected with the adoption of this function. There are very strong reasons for regarding the annulus as a reduced whorl of leaves. Its general appearance and the numerous intermediate forms between leaf-sheath and annulus recorded by Milde (Milde, p. 166) and other observers support this view.

We know of another reduced type of leaf-sheath in *Equisetum*, namely the ochreola of Milde. This structure has been shown to be nothing but the basal whorl of leaves of a branch. The lowest whorl of leaves of each branch appears always to consist of fewer members than the other whorls. Its sheath does not show the commissural furrows so characteristic of the points of junction of the leaves of other sheaths. Duval-Jouve states (p. 67) that the basal sheaths of the branches contain no tracheides, but Milde has shown that its largest tooth, situated on the abaxial side of the branch, may possess a small vascular bundle (Milde, p. 157, and Pl. II, Fig. 36). Here, then, the results of the reduction of an undoubted leaf-sheath appear to be, besides the reduction in number of its members, the loss of vascular elements and the absence of commissural furrows. The general lines on which the reduction of the leafy whorl has proceeded in the annulus and ochreola are strikingly similar. Loss of vascular traces occurs in both, and the absence of commissural furrows in the ochreola may be regarded as the first expression of a process which, if carried farther, might well be expected to lead to an obliteration of the lines of demarcation between the members and to the development of the tissues of the sheath as a homogeneous mass. This is what I believe to have occurred in the annulus.

Perhaps, however, the most important evidence is that derived from the anatomy of the axis near the insertion of the annulus. In my reconstructions of the steles of the cones of *E. arvense*, *E. palustre*, *E. limosum*,

*E. maximum*, *E. hyemale*, and *E. giganteum*. a certain number of parenchymatous meshes are always closed at or near the level of the annulus. Such closure of parenchymatous meshes is of course universal at the nodes bearing leaves. The closure of some meshes is characteristic also of the nodal regions of the cone, though in species with relatively little xylem exceptional nodes occur in which no meshes are closed. We may generalize from these facts and say that the closure of parenchymatous meshes occurs normally only at or near the nodes, while at the vegetative nodes all the meshes are closed. In the light of these facts it will easily be realized that the insertion of the annulus on the axis marks the position of a reduced node. Further, in all the species the anatomy of which is known to us, except *E. giganteum*, a number of new meshes arise above the insertion of the annulus, although the latter is not supplied with traces. It would seem that reduction first affected the formation of traces, secondly the formation of fresh meshes, and lastly the closure of meshes; for the number of meshes formed above the annulus, i. e. above the reduced node, is constantly less, usually markedly less, than the number closed below it. As already pointed out (Browne (1), p. 691), the closure and formation of meshes are not confined respectively to cones showing a decrease or increase in the number of members in the lowest whorl of sporangiophores compared with the number of leaves in the uppermost leafy whorl. These fusions and branchings cannot, therefore, be regarded merely as a method of carrying out such an increase or decrease in the number of axial appendages, but would seem to represent a vestigial nodal character. It might seem strange that in spite of the presence of vascular bundles in its annulus *E. giganteum* is the only species in which no fresh parenchymatous meshes arise between the annulus and the lowest whorl of sporangiophores.<sup>1</sup> This, however, is readily understood if the development of sporangia by the annulus be regarded as a character recently acquired in the phylogeny. The vascular bundles of the annulus would then be a response to the increased need for water, brought about by the development of sporangia. This response would, however, prove a considerable strain on the xylem-producing capacity of the plant in this region. When, as in Cone C, tracheides are actually given off from the axis into the annulus it is clear that there will be less xylem immediately above this point. Now we have seen that one of the results of the reduction of axial xylem at a node of the cone is the failure to produce fresh parenchymatous meshes in the internode above. This is what I believe has occurred at the 'annular node' of *E. giganteum*; for even when the annular bundles die out without reaching the stele their formation imposes a considerable strain on the xylem-producing capacity of

<sup>1</sup> To judge from a single cone of *E. variegatum*, Schleich, of which I have prepared sections, it would seem that in this species no fresh meshes arise above or are closed below the annulus. The cone is small and seems to be reduced, but its structure requires further elucidation.

the plant at this level. In other words, if the production of sporangia and the development of annular bundles as they occur in *E. giganteum* are primitive characters retained by this species, it is strange that just in it the axis above the annulus should have undergone more reduction than in the other species. If, however, the sporangiferous annulus is a phylogenetic innovation, we should expect it to lead to an increase of appendicular xylem, and should be by no means surprised to find that the axis above the annulus had become relatively poor in xylem and that no fresh meshes were formed here.

This, then is the first argument in support of the view that the sporangiferous annulus is not primitive for the genus. Secondly, there is the wider aspect of the question, one difficult to discuss without unduly enlarging the scope of an ordinary anatomical paper. If the annulus was primitively sporangiferous, then it has become normally sterile in the species of *Equisetum* other than *E. xylochaetum* and *E. giganteum*.<sup>1</sup> The annulus would, in fact, be a metamorphosed whorl of sporangiophores, and not a reduced whorl of leaves. On this view the sporangiferous annuli, common abnormalities in certain species, would be explained as reversions, while on the opposite view it would be the cases in which the annulus assumes the characters of a leaf-sheath which would be regarded as reversions (cf. Milde, p. 166). This leads us to the general question of whether leaves and sporangiophores are strictly homologous. In 1903 Professor Bower was led by his advocacy of the 'non-phyllome' theory of the sporangiophores (which he regarded as placental outgrowths) to look upon the annulus of *Equisetum* as a reduced whorl of leaves rather than as a structure transitional between leaves and sporangiophores (Bower, pp. 221 and 241). There is, however, no need to enter here on the general question as to whether fertile and sterile 'leaves' are ultimately strictly homologous or not. For, even if they be referable to a common ancestral, presumably synthetic type of appendicular organ borne by the axis, yet we can safely assume that the highly peculiar form of the Equisetal leafy whorl (though itself an ancient character) is a later development in the phylum than the distinction between sporangiophores and leaves. Consequently, if the annulus represents a primitively sporangiferous whorl its similarity to a reduced whorl of leaves must be due to homoplasy. Certainly as striking, if not more striking, cases of homoplasy are known; yet this explanation of the similarity hardly seems a probable one.

On the view advocated here the annulus is throughout the genus *Equisetum* a reduced whorl of leaves. It is believed that the vascular traces of

<sup>1</sup> Possibly a normally sporangiferous annulus may be found in other species, especially of the group Pleiosteichia of Milde, to which both *E. xylochaetum* and *E. giganteum* belong. In Milde's figures of the cone of the former species no trace of the sporangia is observable from the exterior, and the same thing is true of my cones of *E. giganteum*. Still, on the evidence available, the sporangiferous annulus would not appear to be widely distributed in the genus.

the members of this whorl became obsolete at a relatively early period in the phylogeny of the genus, and that *E. xylochaetum* and *E. giganteum* are descended from forms in which the annulus normally contained no vascular bundles. In fact, though the annulus is a whorl of reduced leaves, the traces found in the two species just mentioned are not regarded as strictly homologous with the traces of the other leaves. Such a view would help us to understand why no fresh parenchymatous meshes are developed above the annulus of *E. giganteum*: the formation of additional vascular elements (the annular bundles) proved a considerable strain on the powers of xylem production, and one result was an insufficiency of xylem above the node to allow of the formation of fresh parenchymatous meshes. On this view, too, we should not be confronted with the difficulty of homologizing the annulus with a whorl of sporangiophores, a difficulty which it is hard to see how to overcome if the annulus be regarded as primitively sporangiferous.

#### SUMMARY.

1. If we were to arrange the cones of the species of *Equisetum*, the anatomy of which is known to us, in a series showing gradual reduction of the vascular system, we should place the species in the following order: (1) *E. arvense*, (2) *E. hyemale*, (3) *E. palustre*, (4) *E. giganteum*, (5) *E. maximum*, and (6) *E. limosum*. In this series, which must not be regarded as a phylogenetic sequence, the first species possesses, relatively to its size, by far the best developed, and the last two species by far the most reduced vascular systems. In the second, third, and fourth species the reduction of xylem has proceeded in somewhat different ways, but, on the whole, the vascular system of the cone has reached much the same degree of reduction.

2. The reduction of the xylem of the cone is manifested in *E. hyemale* and in *E. giganteum*, as in the other species studied, by the persistence of parenchymatous meshes, arising vertically above traces that have departed, upwards into more than one internode, and by their extension laterally above traces given off from at or near the edge of a strand. Both phenomena may be considered to be due to poor development of axial xylem at the nodes of the cone.

3. Specially characteristic of *E. hyemale* and showing relatively good development of the vascular system are the following points: (a) the closure of parenchymatous meshes by the formation of additional tracheides at the node rather than by the oblique course of the tracheides of the branches of a strand above the departure of a trace; (b) the relatively large number of parenchymatous meshes and the high proportion among these of meshes of the first and second orders.

4. Relatively high development of the xylem of the cone of *E. giganteum* is shown: (a) by the slightly greater radial extent of the xylem in this



than in other species in which the anatomy of the cone is known; (b) by the not infrequent development of wide internodal tracts of xylem involving the absence of parenchymatous meshes over median traces; (c) by the fact that closure of parenchymatous meshes more often involves the formation of additional tracheides than the oblique course and ultimate fusion of groups of tracheides lying on either side of the mesh; and (d) by the fact that this fusion of strands, owing to the formation of additional tracheides, not infrequently occurs considerably below the node.

5. Both in *E. hyemale* and in *E. giganteum* the sporangiophores of successive whorls alternate with considerable regularity. But the traces at their insertion on the axial stele do not alternate regularly with those of the whorls above and below. In both species regular superposition occurs when parenchymatous meshes persist unnarrowed on either side of a trace-bearing strand through two or more nodes. This superposition, being due to poor development of axial xylem at the nodes, is less common than in *E. maximum* or in *E. limosum*. Within the species the specimens with less well-developed xylem show more numerous examples of superposition of traces.

6. The traces of the sporangiophores of *E. giganteum* are the most massive yet described for the genus.

7. The traces of the lowest whorls of the cone of *E. hyemale* tend, even when young, to be deflected slightly downwards while passing outwards through the cortex.

8. In *E. hyemale* the axis is narrower at the base than in the middle or slightly above the middle of the cone; but the internodal axial strands and members in a whorl are markedly more numerous at the base of the cone. Consequently the vascular bundles are much closer to one another in the annular region than in the wider parts of the axis of the cone. This probably partly accounts for the relatively high number of meshes closed at or near the level of insertion of the annulus. Above the latter numerous fresh meshes arise.

9. In *E. giganteum* the annulus is normally sporangiferous, the sporangia being attached by their upper ends to the free incurved edge of the annulus. A vascular strand runs to the point of insertion of each sporangium. The number of the latter bears no constant relation to that of the strands in the axis or the lobes of the annulus. The annular bundles may remain free from or be connected with the axial stele; they may branch or remain unbranched.

10. *E. giganteum* differs from the other species studied in that no fresh parenchymatous meshes arise above the annulus. The nodal nature of the axis at the level of the insertion of the latter is, however, supported by the analogy with other species and by the closure of some parenchymatous meshes in this region.

11. The sporangiferous annulus is regarded as derivative in the genus *Equisetum*, and the reasons for this view are briefly examined.

12. In *E. giganteum* the uppermost vegetative node of the fertile branch shows no persistent diaphragm.

In conclusion I wish to express to Professor F. W. Oliver, F.R.S., in whose laboratory these investigations were carried out, my thanks for his encouragement and help, as well as for the material of *E. hyemale* which he placed at my disposal. My thanks are also due to Professor R. C. Maclean, D.Sc., for cones and for a dried specimen of *E. giganteum* collected by him in Chile, and to the authorities at Kew who assisted in the determination of the material of this species.

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#### EXPLANATION OF PLATES VIII AND IX.

Illustrating Lady Isabel Browne's paper on *Equisetum*.

##### PLATE VIII.

Fig. 1. Tangential longitudinal section through the annulus and lower part of the cone of *E. giganteum*. At the base of the section is the annulus, near the edge of which may be seen three annular bundles.  $\times 20$ .

Fig. 2. Radial longitudinal section of the annulus and the lower part of the cone of *E. giganteum*, showing two of the annular bundles and sporangia. The sporangium on the reader's right is partially covered by a sporangiophore which was accidentally bent down during the preparation of the section. The section passes through the point of attachment of the bundle running to the insertion of this sporangium, but just misses that of the bundle corresponding to the sporangium on the reader's left.  $\times 25$ .

Figs. 3, 4, 7, and 9. Transverse sections of axial bundles of *E. giganteum* below the insertion of the annulus. Note the persistent tracheides adherent to the inner side of the carinal canals. The tendency of the carinal canals below the cone to be widest transversely is still to be seen at this level.  $\times 200$ .

Fig. 5. Transverse section of a vascular strand of one of the internodes of the middle part of a cone of *E. giganteum*. Note radial depth of xylem.  $\times 50$ .

Fig. 6. Transverse section of the internodal axis in the upper part of the cone of *E. giganteum*. Note relatively large amount of xylem.  $\times 45$ .

Fig. 8. Transverse section of a node of the cone of *E. giganteum*. Note the comparatively large size of the traces and the absence of a definite central cavity.  $\times 40$ .

Fig. 10. Transverse section of the axial stele of *E. hyemale*, a little way above the insertion of the annulus. Note that a large proportion of the separate bundles of the internode have fused.  $\times 40$ .

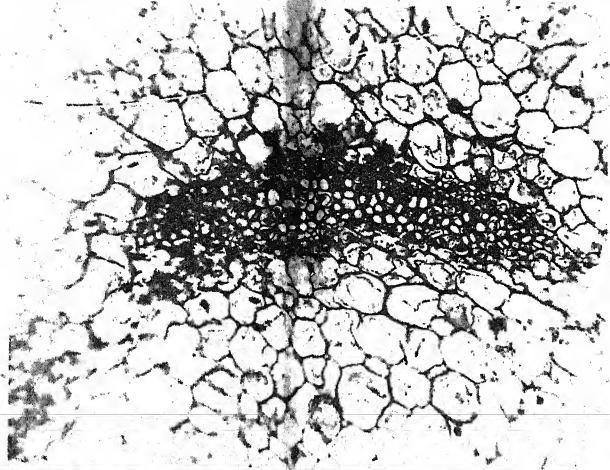
PLATE IX.

Fig. 11. Transverse section of a node of the cone of *E. hyemale*. As the sporangiophores are not all inserted at exactly the same level not all the bundles are giving off traces.  $\times 40$ .

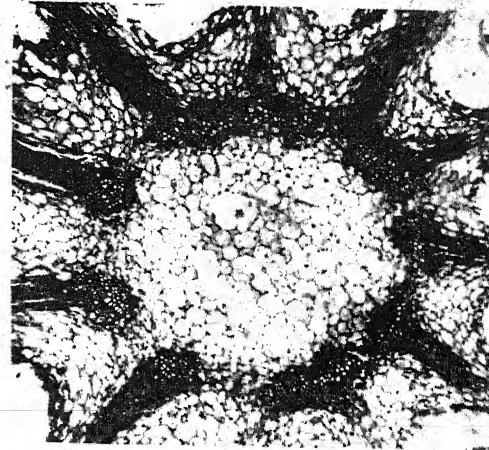
Fig. 12. Transverse section of the internode of the cone in *E. hyemale*.  $\times 55$ .



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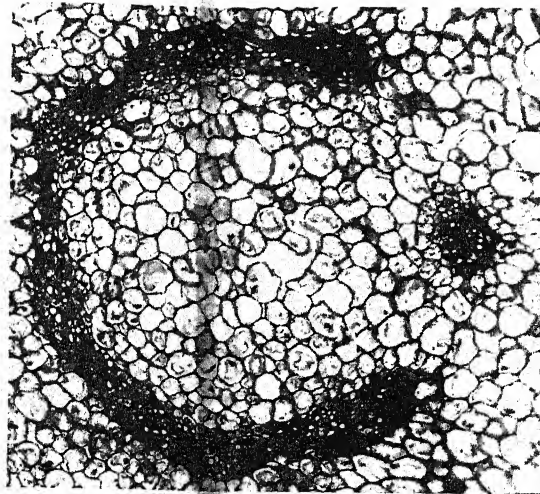
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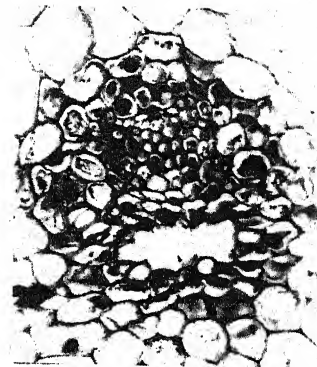
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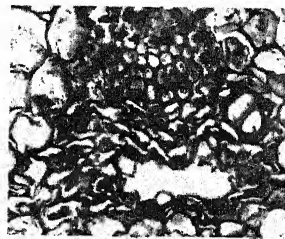
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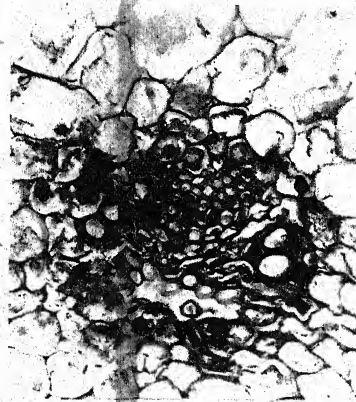
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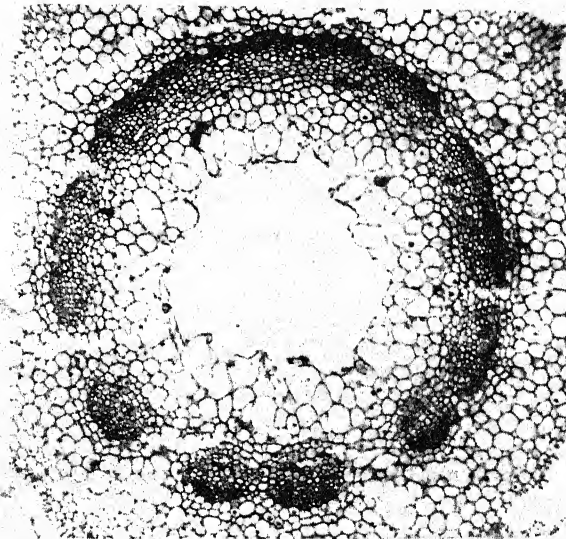
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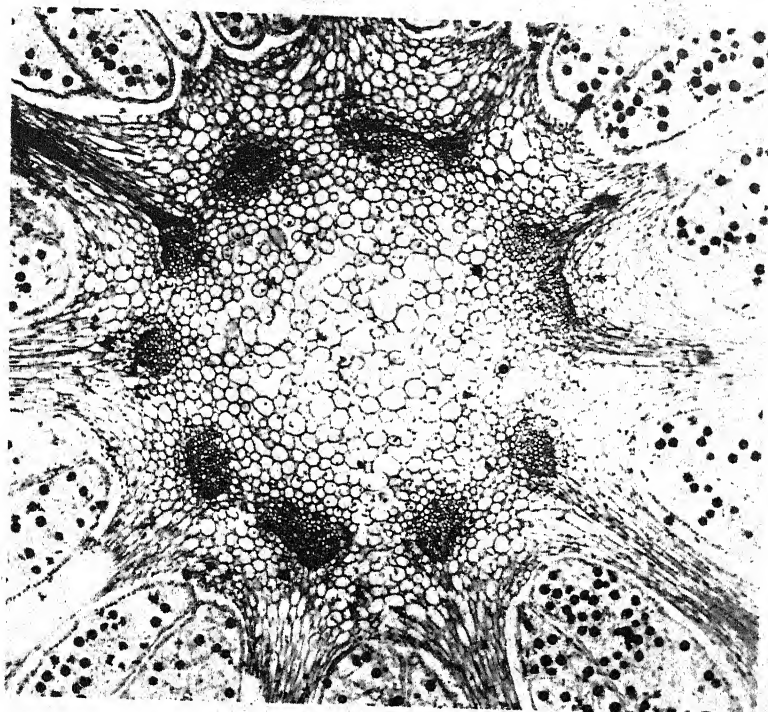
Pittock phot.

BROWNE—EQUISETUM.

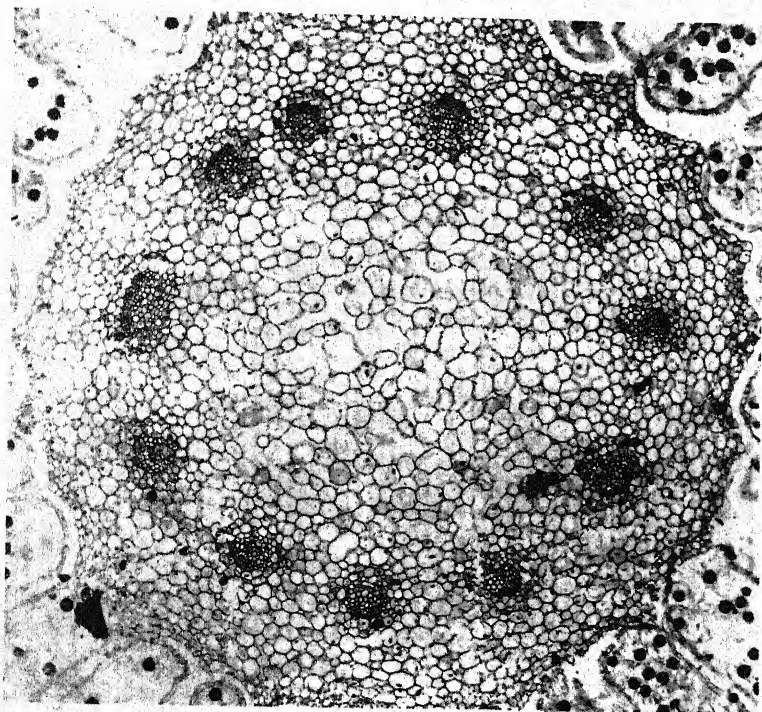
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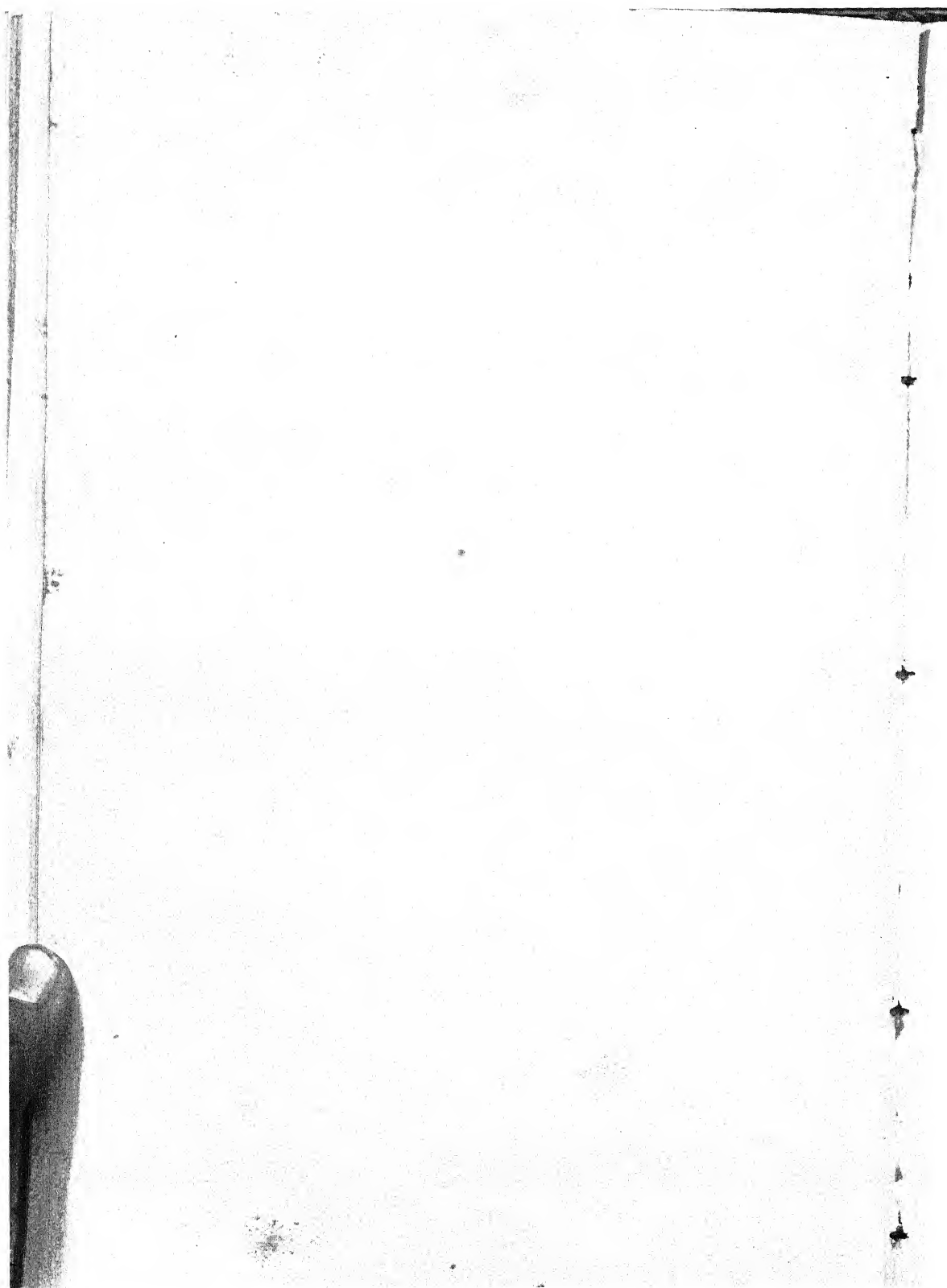
11.



12.

Pittock phot.

Huth coll.





## Studies on the Chloroplasts of Desmids. III.

### X. The Chloroplasts of *Cosmarium*.

BY

NELLIE CARTER, D.Sc.

With Plates X-XIII and two Figures in the Text.

CERTAIN types of chloroplast structure have long been known to occur in the genus *Cosmarium*. A very common form, especially amongst the smaller species of the genus, in which there is a simple four-lobed chloroplast with a single central pyrenoid, was described and figured by Nägeli (1849) and de Bary (1858), as well as certain later investigators.

In a second type, first figured by Nägeli (1849) for *C. margaritifera* and *C. Botrytis*, and later by de Bary (1858), Delponte (1873), and others, there are two chloroplasts in each semi-cell, each with a central pyrenoid and several radiating plates.

Lastly, several species of the genus were known to possess parietal chloroplasts. De Bary (1858) and Delponte (1873) figured such parietal chloroplasts in *C. turgidum*, whilst the latter investigator also indicated a similar condition in *C. ovale*. Klebs (1879) and Gay (1884) also figured parietal chloroplasts in *C. de Baryi* and *C. cucumis* respectively, and Lütkenmüller (1893) investigated the more detailed structure of some of these parietal chloroplasts.

Chloroplasts of all these types were figured in many species by W. and G. S. West (1904-11), and in this work it is also indicated that other types of structure than these three probably occur in the genus, but without carefully stained material it is impossible in most cases to determine the structure of the chloroplasts, and in the majority of species definite information is entirely wanting.

It has long been known that the majority of the genus have axile chloroplasts, and several investigators have considered the comparatively few species known to possess parietal chloroplasts to be worthy of a special genus. Thus Lundell (1871) instituted the sub-genus *Pleurotaeniopsis* to contain all such species, and Lagerheim (1887) raised this to the rank of a genus. Again, Gay (1884) created a new genus *Cosmaridium* to include

*C. cucumis*, a species characterized by parietal chloroplasts. Such classifications based on the structure of the chloroplast were very unwise, particularly since at that time the form of the chloroplasts was known only in comparatively few species, and these had usually only been investigated in the living condition. Thus it is not surprising that several species were wrongly placed. *C. Ralfsii* and *C. elegantissimum*, two species believed by Lundell to possess parietal chloroplasts and placed by him in *Pleurotaeniopsis*, have since been found to be provided with axile ones, whilst in two other species hitherto believed to have axile chloroplasts, *C. Brebissonii* and *C. controversum*, the chloroplasts are now known to be parietal. So that in any case such a division was quite unjustifiable whilst information was so scanty concerning the chloroplasts of the genus.

Again, it has been pointed out by W. and G. S. West (1904-11), that this classification according to the form of the chloroplasts is an unnatural one, resulting in the separation of closely related species. Thus it sometimes happens that chloroplasts of very different forms are found in quite closely related species, e.g. *C. cucumis* and *C. subcucumis*, *C. binum*, and *C. speciosum*.

Parietal chloroplasts seem to have been developed in general in the largest species of the genus, although there are a number of very large species which still retain the axile form. But in these there is a marked tendency to the formation of parietal films of chloroplast. These parietal films were observed in several species (Figs. 21, 37, 56, 69, and 71), but they are frequently very delicate and are difficult to distinguish except after staining. The extent of the parietal layer depends on the profuseness of the chlorophyll. If the chloroplast is fairly massive, then the film will be fairly continuous, whilst if it is scanty there may be just a slight extension of the ridges over the surface of the wall.

Thus it appears that in these large cells the chloroplasts tend to concentrate as much as possible towards the periphery, where they can be of greater help in the process of photosynthesis; but, whilst in some species the chloroplasts have become entirely parietal, others are content to retain the axile form, but mantle the surface of the cell-wall with photosynthetic material, so as to make the best use of their substance. The distinction between parietal and axile chloroplasts is therefore not so clear as was formerly believed to be the case, since in certain axile chloroplasts there may be parietal layers as well. That a sharp line cannot be drawn between parietal and axile chloroplasts is also illustrated by *C. Brebissonii*. Here the chloroplasts are very variable, and whilst in some individuals they may be distinctly parietal, in others they penetrate into all parts of the cell; cf. Figs. 77-81.

Lütkenmüller (1893) drew attention to a peculiar character of the parietal chloroplasts of some species of *Cosmarium*—that the parietal bands

or plates containing the pyrenoids are in some cases covered with tiny outgrowths of chloroplast stretching towards the cell-wall. He found that in *C. tessellatum* these outgrowths were arranged in a definite relation to the warts on the external surface of the wall, each outgrowth being exactly under a wart, but he observed that such outgrowths were also present in species which are not provided with such nodules, e g. *C. turgidum*, *C. de Baryi*. Lütkenmüller was unable to give any explanation for the occurrence of these outgrowths, and raises the question whether they are characteristic of Lagerheim's genus *Pleurotaeniopsis*, observing that he had not been able to ascertain the presence of such outgrowths on the parietal chloroplasts of any species of *Xanthidium*, *Pleurotaenium*, or *Spirotaenia* he had examined. During the present investigation similar outgrowths have been observed in species of *Pleurotaenium*, *Euastrum*, *Xanthidium*, and also in other species of *Cosmarium*, so that this character cannot be considered unique to *Pleurotaeniopsis*. It is possible that this formation of outgrowths is a convenient method of increasing the surface of photosynthetic material exposed to the exterior. It only occurs in chloroplasts which are fairly massive. Where only a delicate film of chloroplast is present such outgrowths never occur, because the whole of the chloroplast is sufficiently exposed to the light. But if there is a fairly thick layer lining the cell-wall, then part of it is projected outwards in the form of outgrowths. Obviously this arrangement is much better than having a simple thick layer of chloroplast. The relation in some species of the outgrowths to the warts on the cell-wall is a rather more difficult problem, since many species having no warts are provided with outgrowths, and in at least one species having warts, *Cosmarium Brebissonii*, the outgrowths are not arranged in any definite relation to these.

With regard to the pyrenoids, there has been some misunderstanding concerning the number of these bodies occurring in the genus. It was formerly believed that, excluding those species having parietal chloroplasts, all other species of *Cosmarium* were provided either with one or two pyrenoids in each semi-cell. The first investigator to show that this rule was not without exception was Lütkenmüller (1893), who reported the presence of unusually large numbers of pyrenoids in *C. pyramidatum* and a few other species.

Later, Duceclier (1917) made further observations supporting those of Lütkenmüller. He also attempted to show that there is some relation between the actual size of the cell and the number of pyrenoids contained in it, examining several species of the genus in order to prove that individuals containing an unusually large number of pyrenoids are often larger in size than individuals of the same species having fewer pyrenoids.

Although it may be true, in general, that large-celled species often have more pyrenoids than the smaller ones, it can scarcely be true in the

consideration of specimens of any one particular species, because of the varying factors in each individual cell. Thus there is considerable variation in the amount of green material contained in different individuals of the same species, irrespective of size, and, again, there is also great variation in the size of the pyrenoids themselves; cf. Figs. 78 and 80. Besides this, in all species of *Cosmarium* which have hitherto been regarded as having one or two pyrenoids in each semi-cell, there is, as in other genera, the possibility of the division of one or both the original pyrenoids to form a group which occupies the same relative position in the cell (Figs. 56, 57, and 69). Thus there is normally the possibility of considerable variation in the number of pyrenoids. The factors which influence the division of the original pyrenoids are very obscure, and cannot simply be external, since the pyrenoids in one part of the cell may divide to form such groups, whilst in other parts of the cell they do not (Figs. 56 and 69). For this reason it is quite impossible to draw any relationship between the number of pyrenoids and the size of the cell.

In the living condition the compact groups of pyrenoids resulting from the division of the original ones cannot be distinguished from single pyrenoids, and so this variability of number has not generally been observed.

The position of the pyrenoids in the axile chloroplasts of *Cosmarium* is dependent on the shape of the chloroplast, and so the points at which they may occur are usually fixed. Thus, although the actual number of pyrenoids is not constant, the number of pyrenoid groups is, since, as a rule, the shape of the axile chloroplast is particularly constant.

Thus the old idea that all species of *Cosmarium* having axile chloroplasts were provided either with one or two pyrenoids, although not strictly true, was not without foundation, for there are many species which, whilst not having invariably either one or two pyrenoids in each semi-cell, yet usually have either one or two groups, each group consisting of one to four pyrenoids, and appearing to have been formed from the division of an original pyrenoid (Figs. 1-23, 28-32, 37-57, and 62-72). The reason that so many species have either one or two points of pyrenoid formation is that two corresponding types of chloroplast structure happen to be very common in the genus. Occasionally there are more than two points of pyrenoid formation in a semi-cell. Thus in *C. pseudoconnatum* there are four (Figs. 35 and 36). It is only very rarely that the pyrenoids do not occur in definite positions in the axile chloroplasts of *Cosmarium*, the only examples encountered during this work being *C. Ralfsii* and *C. ornatum* (Figs. 33, 34, and 58-61). In the former species particularly the pyrenoids are both very numerous and scattered. The opposite is the case with those species having parietal chloroplasts, however, for here it is the rule for the pyrenoids to be indefinite in number and scattered (Figs. 73-88).

The species of *Cosmarium* examined during this investigation included *C. subtumidum*, Nordst., *C. punctulatum*, Bréb., *C. contractum*, Kirchn., *C. crenatum*, Ralfs, *C. depressum*, Lund., *C. caelatum*, Ralfs, *C. speciosum*, Lund., *C. cucurbita*, Bréb., *C. curtum*, (Bréb.) Ralfs, *C. elegantissimum*, Lund., *C. diplosporum*, (Lund.) Lütke., *C. pyramidatum*, Bréb., *C. pseudopyramidatum*, Lund., *C. achondroides*, West, *C. pseudocornutum*, Nordst., *C. ornatum*, Ralfs, *C. Ralfsii*, Bréb., *C. praemorsum*, Bréb., *C. formosulum*, Hoff, *C. binum*, Nordst., *C. margaritifera*, Menegh., *C. Turpinii*, Bréb., *C. pachydermum*, Lund., *C. ochthodes*, Nordst., *C. Botrytis*, Menegh., *C. tetraophthalmum*, Bréb., *C. amoenum*, Bréb., *C. subcucumis*, Schmidle, *C. reniforme*, (Ralfs) Arch., *C. biretum*, Bréb., *C. Brebissonii*, Menegh., *C. cucumis*, Corda, *C. controversum*, West, *C. ovale*, Ralfs, and *C. Askenasyi*, Schmidle. Of these thirty-five species the first thirty have axile chloroplasts, of which twelve have one point of pyrenoid formation per semi-cell, thirteen, two points of pyrenoid formation, whilst in five the chloroplast has an unusual structure. The five last-mentioned species have parietal chloroplasts.

All the species examined having axile chloroplasts have either one or two chloroplasts in each semi-cell. When only one is present it occupies a central position, whilst when there are two they are placed transversely, side by side in the semi-cell. The pyrenoids are usually, though not invariably, embedded in the axis of the chloroplast, often typically one in each, which may give rise to a group of two to four. In a few species more pyrenoids than one are invariably present in each chloroplast, in which case the latter is always unusual in shape. With the exception of *C. Ralfsii* the points at which pyrenoids may occur are nearly always definite and fixed.

The axis of the chloroplast containing the pyrenoids is always provided with a variable number of strands or more definite plates which radiate towards the cell-wall. Their peripheral edges sometimes spread out over the latter, forming more or less extensive parietal layers or films of chloroplast. In a few species these parietal films are the seat of numbers of small proteid granules or naked pyrenoids (Figs. 37-49, 56, and 70). The latter have no relations with the large pyrenoids in the axis of the chloroplast, and are probably formed *de novo* when the conditions are favourable.

*C. subtumidum*, *C. punctulatum*, *C. contractum*, *C. crenatum*,  
and *C. depressum*.

The simplest chloroplasts of the genus were found in these five species, and here, corresponding to the shape of the semi-cell, there is a rather massive axis in the centre, containing usually one pyrenoid (Figs. 3-5, 7, 9, 18-20, and 32). If this original pyrenoid divides, the products of its division may sometimes separate to some little distance, because of the comparatively great width of the axis (Fig. 2), but, although they may not

actually be crowded together, they are nearly always confined to the limits of the axis itself. The axis of the chloroplast gives off four massive lobes which sometimes fork towards the periphery, ending with toothed or smooth edges near the cell-wall (Figs. 9, 20, and 32). In other cases the plates are bent so as to embrace a considerable part of the cell-wall in a parietal manner (Figs. 18 and 20). Very often more than four plates are to be seen, the extra ones being smaller and inserted between the main lobes, stretching towards the front faces of the cell (Figs. 3, 4, 7, 18, and 19). The thickness of the plates varies considerably; in some individuals only sharply defined fringed edges lie against the cell-wall (Figs. 1, 2, 6, 16, and 17), whilst in others the whole chloroplast may be much more massive, and the cell-wall may be almost entirely covered by the broad edges of the ridges (Figs. 8 and 31). Such differences in the character of the chloroplasts are doubtless to be correlated with the amount of stroma starch contained in them.

*C. caelatum* and *C. speciosum*.

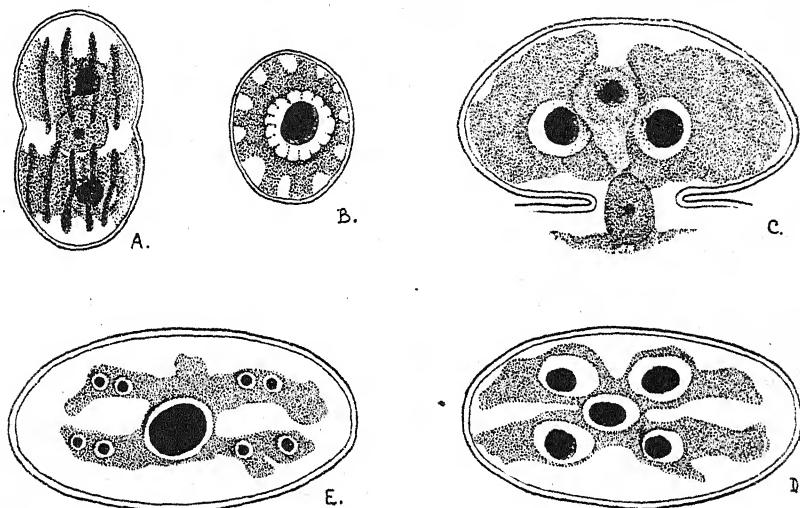
In these two species the chloroplasts only differ from those of the above five species in the rather larger number and more simple form of the plates radiating from the axis. The first-mentioned species has about eight such plates (Fig. 11), whilst in *C. speciosum* there are about ten (Fig. 30). In both cases all the ridges are quite similar to each other, except that those running towards the lateral edges of the semi-cell are naturally larger than those which go towards the front faces. The plates or ridges rarely branch, and end simply on reaching the cell-wall, no attempt at the formation of parietal films of chloroplast being observed (Figs. 10, 28, and 29).

*C. cucurbita* and *C. curtum*.

In those species examined having cylindrical cells the chloroplasts are not very different from those already described. *C. cucurbita* has in each semi-cell a central axis containing usually one pyrenoid, and from this radiate about eight very distinct thin plates, which end near the cell-wall with lobed or fringed edges (Figs. 14 and 15). *C. curtum* has a chloroplast which is quite similar to that of *C. cucurbita*. Its simple axis is usually provided with a single pyrenoid, and there are about eleven plates radiating towards the periphery, ending with sharp edges against the interior of the wall (Text-fig. 1, A and B). Occasionally the ridges were observed to be twisted slightly in a spiral. In these two species, as in all the others dealt with so far, the axis does not always extend right to the apex of the semi-cell, and where this is the case the radiating plates extend beyond it until they reach the extremity of the cell, enclosing a chlorophyll-free space between them; cf. Figs. 1, 14, 16, 17, 28, and 29. This is very pronounced in young semi-cells.

*C. elegantissimum.*

This species seems to have a chloroplast which is quite similar in all essential points to that of *C. cucurbita*, although it was described by Lundell (1871) as having its chloroplasts in the form of parietal bands, and was placed by him in the sub-genus *Pleurotaeniopsis*, together with other species having parietal chloroplasts. Only one specimen was encountered during this investigation, and this had a central pyrenoid in each semi-cell, the chloroplast forming an irregularly shaped mass round it, with several short outgrowths radiating towards the periphery in various directions



TEXT-FIG. 1. A and B, *Cosmarium curtum*, (Bréb.) Ralfs: A, front view; B, end view. C-E, *C. achondroides*, West: C, front view of semi-cell; D, typical optical transverse section; E, optical transverse section of another individual unusual in its pyrenoids. All  $\times 810$ .

(Figs. 12 and 13). The chloroplasts were undoubtedly axile, and it is possible that in individuals with better developed chromatophores more definite plates like those of *Cosmarium cucurbita* might be visible. Possibly Lundell mistook the edges of such plates lying against the cell-wall for parietal bands.

*C. diplosporum.*

In this species the chloroplasts are rather different from those of any of the other species examined having cylindrical cells, being much more elaborate in their structure. There is a large pyrenoid in each semi-cell, embedded in an axile mass of chloroplast. Instead of a limited number of definite plates, numerous thin string-like strands radiate in all directions from the central mass towards the periphery (Fig. 21, lower semi-cell). On reaching the cell-wall the end of each ray becomes flattened and extends in



a parietal manner over its surface, forming an irregularly lobed mass. The whole cell-wall is mantled by these irregularly shaped parietal masses, which form a rough kind of discontinuous network (Fig. 21, upper semi-cell).

*C. pyramidatum* and *C. pseudopyramidatum*.

The chloroplasts of these two species greatly resemble each other, that of the smaller species, *C. pseudopyramidatum*, being the simpler. In the latter species there is a massive axis occupying the centre of the semi-cell, and containing typically one pyrenoid, although sometimes two or more may be present (Figs. 22 and 23). The fact that the central pyrenoid sometimes divides to form a group in this species has been noted by Duce'llier (1917), but, as has already been stated, this character is common to all species of the Desmidiaceae in which the points of pyrenoid formation are definite and fixed. From the lateral and apical edges of the axis there arise a large number of curved plates which are far more irregular in form than those of any other Desmid examined. In the front view their bent edges are seen stretching in various directions, but forming a single series which encircles the central pyrenoid or group of pyrenoids (Fig. 22), whilst in the end view they are seen to be arranged in four vertical lines (Fig. 23). The edges of the bent plates lying against the cell-wall are cut into finger-like projections which extend in various directions over its surface.

*C. pyramidatum* (Fig. 24) has curved or bent plates which are exactly similar to those of *C. pseudopyramidatum*, the only differences between the chloroplasts of the two species being in the shape of the axis and the arrangement of the pyrenoids (Fig. 24). In the larger species there is a very thin plate, rather triangular in shape, in the middle of the semi-cell, and all round the edges of this the bent plates arise, travelling towards the front faces of the semi-cell (Fig. 24). In the end view, as before, they are seen to be arranged in four main series (Fig. 27). The pyrenoids are very variable in number (2-14), and are arranged in a triangular or horseshoe-shaped series in the rather thicker part of the chloroplast where the bent plates arise from the thin axis (Figs. 24 and 25). Usually the pyrenoids all lie in one plane parallel to the front faces of the semi-cell (Fig. 25), but when they are very numerous they seem to separate into two different planes in the bases of the plates themselves (Figs. 26 and 27). Perhaps the most common number of pyrenoids is three, arranged one at each corner of the triangular axile plate (Fig. 24), but, as was noticed by Lütke'müller (1893) and Duce'llier (1917), they are very variable. Occasionally only one pyrenoid is present in the central position, as is the case with *C. pseudopyramidatum*, but such cases are rare, the axis in the middle of the cell being usually far too thin for the accommodation of pyrenoids.

Lütke'müller stated that when three or more pyrenoids are present in *C. pyramidatum* it very often happens that a rounded hole appears in the

centre of the chloroplast between them. He thought that this rupture was brought about by the travelling towards the periphery of the products of division of an original central pyrenoid, a single pyrenoid being, according to his theory, general at first in all individuals. Whether or not this is so could not be ascertained without the examination of actively dividing material, but considering the behaviour of the chloroplasts of other species during cell-division it seems most likely that, since there are nearly always two pyrenoids at the base of the semi-cell, either these, or possibly in some cases the products of their division, would enter the new semi-cell when cell-division took place, whilst in the case of individuals containing as many as fourteen pyrenoids in a half-cell, the new semi-cell would doubtless be supplied with quite a number of pyrenoids. It is most likely, therefore, that the semi-cells of *C. pyramidatum* often contain originally at least two pyrenoids, in which case the travelling apart of the products of division of an original central one could not explain the formation of the central hole. Although it was observed that the axis of this species is always very delicate, consisting sometimes merely of a delicate reticulum, a cavity such as was described by Lütkenmüller was never noticed in such material as was available. It is possible that such a delicate structure could not be detected in the living condition, and that this accounts for Lütkenmüller's statement.

Ducellier (1917) reports that one collection of this species examined by him contained quite a large proportion (30 per cent.) of individuals having only one pyrenoid in a semi-cell. This may have been a special character developed in that particular locality, for it is quite possible that occasionally in newly-divided specimens the young semi-cell is only provided with one pyrenoid, especially in those forms containing very few pyrenoids. In Ducellier's collection there were never more than two pyrenoids in each semi-cell. It must be remembered that the chloroplast has to push its way from the old semi-cell through the comparatively narrow isthmus into the young semi-cell after cell-division, and therefore one must not be surprised if distortions sometimes occur. Thus it is quite possible that, following such an abnormal division, the young semi-cell is found to be provided with only one pyrenoid, and repeated division might result in the production of quite a large proportion of such individuals containing only one pyrenoid in a semi-cell, as in Ducellier's collection. Nevertheless it seems true that as a rule there are two or more pyrenoids in the chloroplasts of this species, and that the axis of the chloroplast is usually very delicate, so that the actual centre of the semi-cell is usually destitute of pyrenoids.

*C. achondroides.*

This species was originally described and figured by West (1909) as having parietal chloroplasts. A subsequent examination of stained specimens has shown, however, that there is actually one axile chloroplast in each

semi-cell which is somewhat similar in form to those of *C. punctulatum*, *C. depressum*, &c., but differs from these in the arrangement of the pyrenoids. As in the simple chloroplasts of these small species, there is a central axis containing usually one pyrenoid (Text-fig. 1, C), but this may occasionally bud off others. From this axis four lobes are given off, which expand to form somewhat massive parietal plates, two of which lie against each front face of the semi-cell (Text-fig. 1, C). Commonly there is a large pyrenoid in each of these lobes, equal in size to the central pyrenoid, so that the typical number of pyrenoids is five (Text-fig. 1, D). Those in the lateral lobes of the chloroplast, however, are not so constant as the central one, and occasionally one or more of the lobes may be entirely free from them, whilst in others, if present, the pyrenoids are very reduced in size, and look as if they had been formed *de novo*. In one semi-cell at least eight such tiny pyrenoids were observed in these lateral lobes (Text-fig. 1, E). The most common type, however, had a large pyrenoid in each of the four lobes in addition to the central one, and in every individual examined at least one semi-cell showed this structure.

*C. pseudoconnatum.*

The chloroplast of this species is rather different from that of any other species examined. It was originally believed to have parietal chloroplasts, but is mentioned by Lütkenmüller (1893) as being the only species included in *Pleurotaeniopsis* in spite of the fact that it has an axile chloroplast, because it was known to have four pyrenoids in each semi-cell, whilst in *Cosmarium* proper there were only supposed to be one or two.

The chloroplast of *C. pseudoconnatum* consists of four large wedge-shaped masses arranged symmetrically in each semi-cell, each mass containing typically one pyrenoid, and all four being united in the interior by their thin ends (Fig. 36). Towards the periphery the broad external surface of each mass is grooved to form irregular ridges, whose edges spread out over the cell-wall (Fig. 35).

*C. ornatum* and *C. Ralfsii*.

*C. ornatum* and *C. Ralfsii* have chloroplasts which show some similarities whilst being different from those of any other species examined. The smaller species, *C. ornatum*, has an axile chloroplast in each semi-cell, consisting of a relatively short broad axis, containing two or three pyrenoids, with about eight plates radiating in various directions towards the periphery (Figs. 33 and 34).

The larger species, *C. Ralfsii*, was for a long time supposed to have parietal chloroplasts, and was placed by Lundell (1871) in his sub-genus *Pleurotaeniopsis*, together with other species having parietal chloroplasts. Lütkenmüller (1910) corrected this mistaken idea by means of a short note and figure. The chloroplast in this species consists of a broad axis occupying

the middle of the semi-cell, from which a number of branching ridges radiate towards the periphery (Fig. 59). The branching and spreading out of the ridges near the cell-wall result in the formation of broad peripheral masses of chloroplast, which in the living condition doubtless give the impression of parietal bands (Figs. 59 and 61). The ultimate branches of the ridges end against the cell-wall with smooth or fringed edges. The pyrenoids vary considerably in size and number (6–14), and are embedded in the thicker parts of the chloroplast, either where the ridges arise from the axile plate, or nearer the periphery, at the points of branching of the ridges themselves (Fig. 61).

There is a strong tendency in this species towards the hollowing away of the axile plate of the chloroplast in the apical region of the semi-cell, exactly similar to that observed in the thicker-celled species of *Micrasterias*.<sup>1</sup> The chlorophyll-free portion is often quite small, but in some individuals it extends right as far as the nucleus, and consequently in such cases there are two chloroplasts in each semi-cell instead of one; cf. Figs. 59, 60, and 58. The general structure of the chloroplast, and its variations in the median region of the cell, together with the arrangement and variation in number and size of the pyrenoids, are all very suggestive of the thicker-celled species of *Micrasterias*; in fact it is scarcely possible to distinguish any differences at all between the chloroplasts of this species and *M. oscitans* in transverse section. Moreover, its chloroplast is quite different from that of any of the other species of *Cosmarium* examined, with the possible exception of the small species *C. ornatum*. There are some similarities between the chloroplasts of the latter species and those of *C. caelatum* and *C. speciosum*; cf. Figs. 33, 10, and 28; but in the irregular form of its ridges and scattered pyrenoids it agrees more nearly with *C. Ralfsii*. In *C. ornatum* there are almost invariably two or three pyrenoids, and they never give one the impression that they might have been formed by the division of an original central one. Thus we may assume that in *C. ornatum*, as in *C. Ralfsii*, there is not merely one point of pyrenoid formation.

The seventeen species dealt with so far possess normally one chloroplast in each semi-cell. The thirteen species which will now be described have two chloroplasts transversely disposed in each semi-cell. In each case the chloroplast has a funnel-shaped axis arising on one side of the nucleus, with which it is in close connexion, and this axis stretches towards the corresponding lateral region of the semi-cell (Fig. 47). It increases in thickness as it passes upwards, and finally ends about half-way between the apex of the semi-cell and the nucleus in a swollen head which contains the pyrenoid or group of pyrenoids (Fig. 47). Two such axes arise, one on each side of the nucleus, and they lie symmetrically, one on each side of the median line.

<sup>1</sup> Vide Carter, N.: Studies on the Chloroplasts of Desmids. II. Ann. Bot., vol. xxxiii, 1919.

The axis is not usually very large, and if more pyrenoids than one are present they usually cause much distortion (Figs. 57 and 69). From the axis of each chloroplast a number of plates stretch out towards the cell-wall (Figs. 38-49), or in some cases, instead of definite plates, numerous thin strands radiate in all directions towards the cell-wall (Figs. 62-70). In practically every species the edges of the plates or the ends of the strands spread out over the internal surface of the cell-wall to form greater or smaller parietal extensions, but this is doubtless a feature which is subject to considerable individual variation (Figs. 37, 50, 54, 56, 67, 69, and 71).

*C. formosulum.*

The axis of the chloroplast in this species is provided with about four simple plates which radiate towards the periphery (Text-fig. 2, B), their edges spreading irregularly over the surface of the wall, but only covering a comparatively small area of its surface (Text-fig. 2, A and B).

*C. binum.*

This species, which is frequent in the tropics, has chloroplasts which are quite similar to those of *C. formosulum*, but the radiating plates are more massive, and there is some attempt at branching, relatively more of the surface of the cell-wall being covered by their spreading extremities (Figs. 54 and 55). The shape of the cell-wall apparently influences the development of the chloroplast to some extent in this species, for the broad edges of the four lateral plates lying under the marginal crenations of the cell-wall are produced to form four series of short radiating ridges, each ridge corresponding to one of the crenations (Fig. 54). This relation between the cell-wall and chloroplasts recalls the condition described by Lütkenmüller (1893) in the parietal chloroplasts of *C. tessellatum*, &c.

*C. margaritifera.*

The plates radiating from the axis in this species are much more irregular in form than in the two previous species (Fig. 53). In the front view of the cell, their widened extremities are seen running in all directions over the surface of the cell-wall (Fig. 52), and they are frequently seen to branch (Fig. 53).

*C. pachydermum.*

The axis of each chloroplast is provided with about five radiating plates (Fig. 72), the edges of which, on reaching the cell-wall, spread out on either side to form finger-like outgrowths, sometimes of considerable length and often branching. These flattened processes are closely adherent to the surface of the wall, and so in the front view the cell is seen to have several irregularly branching strings of parietal lobes of chloroplast (Fig. 71). The

plates may previously fork before spreading out to form the parietal outgrowths, but probably part of the branching visible in the optical transverse section (Fig. 72) is only apparent, being due to the lateral extension of the outgrowths on both sides of the plate. The actual shape of the outgrowths varies amongst individuals, and the extent of their development depends largely on the size of the chloroplast. In some individuals with slender chloroplasts the edges of the plates form merely a delicate network against the cell-wall, and the beautiful lobing so conspicuous in others is wanting.

*C. Turpinii*, *C. ochthodes*, *C. Botrytis*, and *C. tetraophthalmum*.

These species have chloroplasts which are very similar to each other. The axis of each chloroplast gives rise to four to six plates which radiate towards the exterior (Figs. 40, 57, and Text-fig. 2, H), and the peripheral edges of these plates often spread out over the internal surface of the cell-wall to form extensive parietal films of chloroplast in the form of a delicate reticulum (Figs. 37, 45, 49, 56, Text-fig. 2, G). Very often practically the whole surface of the wall is thus mantled with a thin layer of photosynthetic matter, only small gaps being left. In *C. ochthodes* this parietal layer is often the seat of numerous tiny proteid granules, or naked pyrenoids (Figs. 37 and 49), and these have also been occasionally noticed in *C. tetraophthalmum* (Fig. 56). The ordinary large pyrenoids of the latter species show a particular tendency to multiply, and a group of two to four is commonly found embedded in the axis of one or more of its chloroplasts (Figs. 56 and 57).

*C. amoenum*, *C. subcucumis*, *C. reniforme*, and *C. biretum*.

In the chloroplasts of these species there are simple strands radiating from the axis rather than definite plates. The rather spherical mass of chloroplast containing the pyrenoid or group of pyrenoids sends out these projections towards the cell-wall in all directions, except towards the other chloroplast of the semi-cell (Figs. 62 and 70). One of these outgrowths usually connects up the axis of the chloroplast with the nucleus (Fig. 62). The chloroplast strands are very numerous, and may sometimes branch slightly (Figs. 64 and 66). On reaching the cell-wall they usually spread out to form irregularly shaped parietal masses lying against the cell-wall, forming a rough kind of network, which is, however, rather different from the more or less continuous parietal reticulum of *C. ochthodes* (Figs. 67 and 69).

The chloroplasts of the specimen of *C. amoenum* examined (Figs. 62 and 63) were not sufficiently massive to show the formation of a parietal network, but it is quite likely that under favourable conditions it is also developed in this species.

In the case of *C. biretum* it was quite impossible to trace the parietal spreading of the ends of the chloroplast strands owing to the dense staining of the cell-wall itself, the chloroplasts being investigated entirely from sections (Figs. 64–6).

The pyrenoids of *C. reniforme* show a marked tendency to multiply, as many as three being commonly packed together in the axis of a single chloroplast (Fig. 69), whilst in several individuals of this species the small pyrenoids or proteid granules so common in *C. ochthodes* were also observed in the parietal parts of the chloroplast (Fig. 70).

*C. praemorsum.*

Examples of this species from Dartmoor were found to have chloroplasts almost exactly similar to those of *C. formosulum*, there being two axile chloroplasts in a semi-cell, each with a single pyrenoid. There was very little attempt at the formation of parietal films of chloroplast (Figs. 50 and 51).

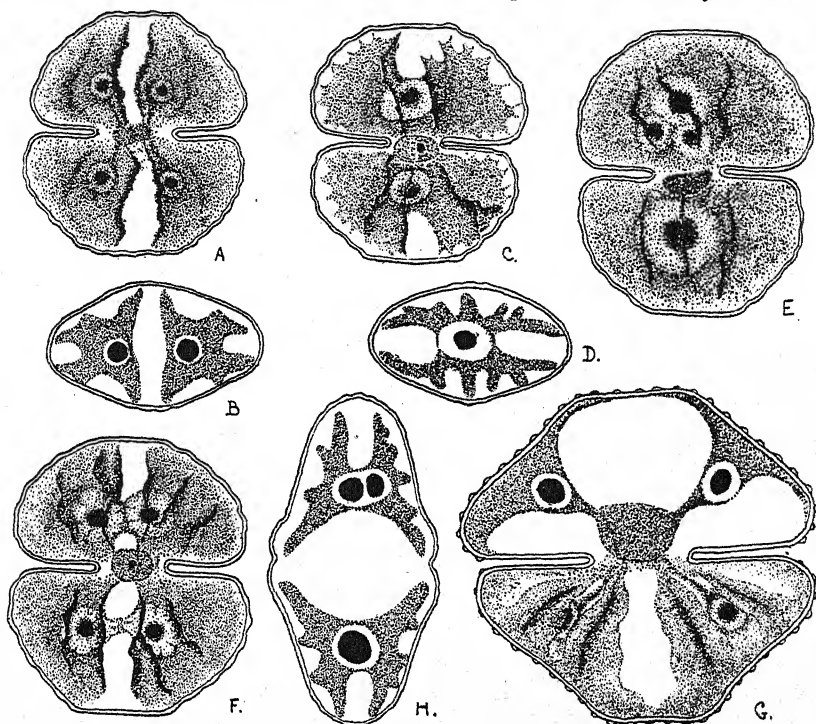
Later, a collection of algae from a tank in the Botanical Department of the University of Bangor was provided by Dr. Acton, which contained, amongst others, two forms of *Cosmarium*, which, although differing slightly from each other, are both to be referred to the species *C. praemorsum*. The smaller of the two was typical *C. praemorsum*, and, like the other examples previously examined, contained two chloroplasts in each semi-cell. The other form was slightly larger, and differed from the first in its slightly projecting apices, whilst its chloroplasts were quite different (Text-fig. 2, C, E, and F). Here there was only one chloroplast in each semi-cell, consisting of an axis containing typically one pyrenoid, and several plates radiating towards the periphery (Text-fig. 2, C and D). The axis of the chloroplast was often shortened in the apical region of the semi-cell (Text-fig. 2, C).

A conspicuous character of this form was the unusual way in which the division of the central pyrenoid affects the chloroplast. Sometimes an individual was encountered having a compact group of two or three pyrenoids in the centre of the semi-cell, as one would naturally expect (Text-fig. 2, E). But frequently there was a tendency for the two pyrenoids resulting from the division of the original one to separate from each other, and also for the chloroplast itself to constrict into two halves at the same time (Text-fig. 2, F). The union of the two parts of the chloroplast was always retained, however, even if only by means of a thin string-like strand connecting the two pyrenoids (Text-fig. 2, F, lower semi-cell). Sometimes even the starch-grains in the starch-sheaths of the pyrenoids were distorted by the pulling apart of the two halves of the chloroplast. Two separate chloroplasts were never observed, however, and so these variations in the form of the chloroplast are very difficult to explain. Because of the slightly different shape of the cell, and the fact that there is only one chloroplast in



each semi-cell, this particular form may be a distinct variety of *C. praemorsum*. At any rate it is interesting to note that two different kinds of chloroplast structure may sometimes occur in the same species.

The remaining five species of *Cosmarium* examined were provided with parietal chloroplasts, which lie against or near the surface of the cell-wall. In *C. Brebissonii* the disposition of the chloroplasts is not always distinctly



TEXT-FIG. 2. A and B, *C. formosulum*, Hoff: A, front view; B, optical transverse section. C-F, *C. praemorsum*, Bréb.: C, front view; D, end view; E and F, showing irregularities in the number of pyrenoids. G and H, *C. Turpinii*, Bréb.: G, front view, upper semi-cell in optical section; H, optical transverse section. All  $\times 810$ .

parietal owing to the irregular distending of the chloroplasts in all directions by the large and numerous pyrenoids (Figs. 75-81). The number of chloroplasts is often four, but they are subject to variation, especially in *C. Brebissonii* (Figs. 75-81), whilst in size and number the pyrenoids also vary considerably, often becoming very numerous in the larger species (Figs. 82-5). This variation in number of the chloroplasts and pyrenoids is general with parietal chloroplasts.

Projections on the surface of the parietal expansions of chloroplast such as were described by Lütkenmüller (1893) in *C. tessellatum*, &c., have been observed in the chloroplasts of four of the five species examined having parietal chloroplasts.

*C. Brebissonii.*

This species has most peculiar and variable chloroplasts which are quite different from those of any other species examined, and it is difficult to decide whether they should be considered axile or parietal. The general appearance varies considerably according as the chloroplast is massive or only very feebly developed, and its disposition apparently depends on this as well (Figs. 75-81). In specimens which have only feebly developed chloroplasts these take the form of thin parietal bands with small embedded pyrenoids (Fig. 78). The number of chloroplasts is about four, but is variable, and they are in close connexion with the nucleus at the base of the semi-cell, whilst their lateral margins are variously lobed or toothed. Sometimes the parietal bands are connected together in various ways by means of thread-like strands traversing the interior of the cell (Fig. 77). Specimens having this structure are comparatively rare.

In the majority of cases the chloroplasts are more massive and are removed to some little distance from the cell-wall. In a transverse section many specimens show a peripheral series of about five chloroplasts just within the cell-wall, each containing one or more pyrenoids which project inwards towards the centre of the cell, whilst the thin film of chloroplast surrounding them sends out numerous finger-like outgrowths towards the cell-wall (Fig. 79). In such cases, the chloroplasts, although projecting a fair distance into the interior of the cell, can scarcely be called axile.

Finally, in specimens having very massive chloroplasts, a transverse section shows an irregular central mass of pyrenoids surrounded by a thin film of chloroplast, which towards the periphery seems to associate itself into several more or less distinct lobes which send out long finger-like projections to the cell-wall (Figs. 80 and 81). From the exterior such individuals are extremely dense and the nature of their contents cannot be elucidated. The pressure of one lobe of the chloroplast against its neighbour causes the formation of several longitudinal ridges, and this is all that can be distinguished in whole specimens.

Although the chloroplast of such specimens seems to be intact and solid in the middle of the semi-cell, towards the apex and base the lobes seem to become more distinct and free from each other, and here the chloroplast is more definitely parietal. Besides this, it often happens that in transverse section one lobe is seen to be quite free from the remaining solid mass of chloroplast and pyrenoids (Fig. 81).

Taking into account all these points it seems wiser to consider that the chloroplast of *C. Brebissonii* consists of four to seven parietal parts. Each part begins as a thin strand near the nucleus and proceeds towards the apex of the semi-cell, spreading out laterally just beneath the cell-wall so as to screen, by the united efforts of all the chloroplasts, practically the whole

surface (Fig. 75). Each chloroplast contains one to four pyrenoids, whilst its edges and external surface are usually covered with numerous tiny finger-like outgrowths which stretch towards the cell-wall (Figs. 75 and 76). These outgrowths do not appear to have any definite relations with the granules on the external surface of the cell-wall, and sometimes become extraordinarily large, taking the form of short ridges running longitudinally. Sometimes a small chloroplast arises near the nucleus and stretches upwards, hidden in the interior of the cell, finally emerging near the apex of the semi-cell to form a parietal mass (Figs. 75 and 76). In cases where the pyrenoids become very numerous they cause extreme distortion of the chloroplasts, spreading farther and farther into the interior of the cell until the chloroplasts of opposite sides meet, and a practically solid mass of pyrenoids and chloroplasts is formed (Fig. 80 and 81).<sup>1</sup>

*C. cucumis.*

It was only possible to examine a few specimens of this species, and in all these there was a thin parietal layer of chloroplast covering the whole cell-wall in the form of a delicate reticulum. Occasionally a number of more or less distinct bands, running longitudinally, could be distinguished (Fig. 73), but it usually happened that the parietal film was quite evenly distributed, with only occasional irregular colourless spaces. The chloroplast was in all cases closely adherent to the cell-wall, and the pyrenoids, which were about fourteen to twenty in number and very variable in size, were suspended at some little distance in the interior of the cell by a delicate network of chloroplast (Fig. 74). In the limited number of specimens examined this delicate structure was general, and in no case were projections present on the external surface of the chloroplast. It is possible, however, that, had other material been available, specimens having more massive chloroplasts might have been encountered, and perhaps in such individuals the tiny outgrowths observed in the other species of the genus having parietal chloroplasts might be present.

*C. controversum.*

This species was originally described as having axile chloroplasts with two pyrenoids in each semi-cell.<sup>2</sup> Its chloroplasts are parietal, however, four being the usual number (Fig. 86). Occasionally there are only two chloroplasts, one on each front face (Fig. 87), and possibly more than four may also sometimes occur. The chloroplast plates may be of some thickness and contain from one to five pyrenoids (Figs. 86-8). They are removed to

<sup>1</sup> The pyrenoids also similarly distend the chloroplasts in certain species of *Xanthidium* having parietal chloroplasts, and here, too, the pyrenoids of opposite faces of the semi-cell are sometimes nearly incontact. See Carter, N.: *Studies on the Chloroplasts of Desmids.* I. *Ann. Bot.*, vol. xxxiii, 1919.

<sup>2</sup> Vide West, W. and G. S. (1904-11), vol. iv, p. 9.

some little distance from the cell-wall, and their edges and external surfaces are covered with outgrowths which project towards the periphery, each projection corresponding to one of the granules on the cell-wall, as described by Lütkenmüller (1893) for *C. tessellatum* (Figs. 86-8).

*C. ovale* and *C. Askenasyi*.

In these two species there are usually four parietal chloroplasts in each semi-cell, two on each front face (Fig. 82), although occasionally three may be present on one or other of the faces (Figs. 84 and 85). The main part of each chloroplast consists of a thin layer removed to some little distance from the cell-wall, and containing from six to twelve pyrenoids. In both species there are numerous tongue-like projections stretching from the external surfaces of the plates towards the periphery, those of *C. Askenasyi* being rather more delicate and relatively more numerous than those of *C. ovale* (Figs. 82-5).

SUMMARY OF THE SPECIAL CHARACTERS OF *COSMARIUM*.

Most of the species of *Cosmarium* examined have axile chloroplasts, although in a few the chloroplasts were parietal. In those species having axile chloroplasts there are either one or two chloroplasts in each semi-cell, and very often there is typically one pyrenoid in the axis of each chloroplast.

The statement that all species of the genus having axile chloroplasts possess either one or two pyrenoids in a semi-cell is untrue, although there are many species which have either one or two points of pyrenoid formation. As in other genera of the Desmidiaceae, the actual number of pyrenoids present depends on the individual itself, and at any time a group of pyrenoids may be formed where originally there was only one.

Many of the smaller species have a single chloroplast in each semi-cell consisting of a central axis, containing typically one pyrenoid, from which radiate either four more or less forked plates or a number of simple ridges or string-like outgrowths.

In *C. diplosporum* there is a rough kind of parietal network arising from the lateral expansion of the ends of the chloroplast rays extending from the central axis.

The plates arising from the central axis containing the central pyrenoid or group of pyrenoids are in *C. pseudopyramidatum* very complicated in form and irregular in arrangement.

*C. pyramidatum* is similar to the previous species in the form and arrangement of its plates, but differs in its more delicate axis and in the arrangement of its pyrenoids, which, unlike those of *C. pseudopyramidatum*, rarely occupy the centre of the chloroplast.

*C. achondroides* differs from the first type in that the lateral lobes of its chloroplast are provided with pyrenoids as well as its central axis.

In *C. pseudoconnatum* the chloroplast is axile with four wedge-shaped masses radiating towards the periphery, each mass possessing typically one pyrenoid.

*C. ornatum* and *C. Ralfsii* differ from all other species examined having axile chloroplasts in their scattered pyrenoids. There are rarely more than three in the former species, but in the larger one they are much more numerous. The chloroplast of *C. Ralfsii* shows striking resemblances to those found in certain thick-celled species of *Micrasterias*.

Many species of *Cosmarium* have two axile chloroplasts in each semi-cell, there being one point of pyrenoid formation in each chloroplast. The axis which contains the pyrenoid or group of pyrenoids is surrounded by a number of radiating plates, or more numerous string-like projections, whose peripheral edges in many cases spread out over the internal surface of the cell-wall, either in irregular parietal masses, or as a more or less continuous reticulated film.

Two forms of *C. praemorsum* were examined, containing one and two chloroplasts in a semi-cell respectively.

*C. Brebissonii* was found to have most peculiar and variable chloroplasts, which are sometimes parietal, but in other individuals penetrate into all parts of the cell.

In a few species the chloroplasts were found to be entirely parietal, with scattered pyrenoids. In such species the number of chloroplasts and pyrenoids present is variable.

In conclusion, I wish to express my gratitude to the Birmingham Natural History Society, and also to the Royal Society, for grants to help in the cost of reproducing the plates illustrating this work. I have also to acknowledge the invaluable help which I received during this investigation from the late Professor G. S. West, who not only identified all the species examined, but also provided some of the material.

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## DESCRIPTION OF PLATES X-XIII.

Illustrating Dr. Nellie Carter's paper on the Chloroplasts of Desmids.

During the prolonged processes of preparation the specific characters of Desmids are often obliterated, but all the species examined were identified, either in the living or carefully fixed condition, by the late Professor G. S. West.

PLATE X. (All  $\times 810$ .)

Figs. 1-5. *Cosmarium subtumidum*, Nordst. Figs. 1 and 2, front view; Figs. 3-5, various transverse sections.

Figs. 6 and 7. *C. crenatum*, Ralfs. Fig. 6, front view; Fig. 7, optical transverse section.

Figs. 8 and 9. *C. contractum*, Kirchn. Fig. 8, front view; Fig. 9, optical transverse section.

Figs. 10 and 11. *C. caelatum*, Ralfs. Fig. 10, front view; Fig. 11, optical transverse section.

Figs. 12 and 13. *C. elegantissimum*, Lund. Fig. 12, front view; Fig. 13, optical transverse section.

Figs. 14 and 15. *C. cucurbita*, Bréb. Fig. 14, front view; Fig. 15, transverse section.

Figs. 16-20. *C. punctulatum*, Bréb. Figs. 16 and 17, front view; Figs. 18-20, transverse sections.

Fig. 21. *C. diploporum*, (Lund.) Lütke., front view, lower semi-cell in optical section.

Figs. 22 and 23. *C. pseudopyramidatum*, Lund. Fig. 22, front view; Fig. 23, optical transverse section.

Figs. 24-7. *C. pyramidatum*, Bréb. Fig. 24, front view; Fig. 25, side view; Fig. 26, optical longitudinal section of a large specimen containing numerous pyrenoids; Fig. 27, optical transverse section of a similar individual.

Figs. 28-30. *C. speciosum*, Lund. Fig. 28, front view; Fig. 29, side view; Fig. 30, optical transverse section.

Figs. 31 and 32. *C. depressum*, Lund. Fig. 31, front view; Fig. 32, optical transverse section.

Figs. 33 and 34. *C. ornatum*, Ralfs. Fig. 33, front view; Fig. 34, optical transverse section.

Figs. 35 and 36. *C. pseudoconatum*, Nordst. Fig. 35, front view; Fig. 36, transverse section.

PLATE XI. (All  $\times 810$ .)

Figs. 37-49. *C. ochthodes*, Nordst. Fig. 37, front view; Figs. 38-41, serial transverse sections from the apex of the semi-cell to the sinus; Figs. 42-4, serial longitudinal sections, parallel to the lateral faces of the cell, through one half of the semi-cell and one chloroplast only; Figs. 45-9,

serial longitudinal sections parallel to the front faces of the semi-cell (*s*, starch grains of the sheaths of the pyrenoids).

Figs. 50 and 51. *C. praemorsum*, Bréb. Fig. 50, front view; Fig. 51, optical transverse section.

Figs. 52 and 53. *C. margaritifera*, Menegh. Fig. 52, front view; Fig. 53, transverse section.

Figs. 54 and 55. *C. binum*, Nordst. Fig. 54, front view; Fig. 55, optical transverse section.

Figs. 56 and 57. *C. tetraophthalmum*, Bréb. Fig. 56, front view; Fig. 57, optical transverse section.

#### PLATE XII.

Figs. 58-61. *C. Ralfsii*, Bréb.  $\times 510$ . Figs. 58-60, various individuals in front view; Fig. 61, transverse section.

Figs. 62 and 63. *C. amoenum*, Bréb.  $\times 810$ . Fig. 62, front view; Fig. 63, optical transverse section.

Figs. 64-6. *C. biretum*, Bréb.  $\times 810$ . Fig. 64, longitudinal section parallel to front faces of the cell; Fig. 65, transverse section; Fig. 66, longitudinal section parallel to the lateral faces of the cell (contents of lower semi-cell not shown).

Figs. 67 and 68. *C. subcucumis*, Schmidle.  $\times 810$ . Fig. 67, front view (right-hand chloroplast of lower semi-cell in optical section); Fig. 68, optical transverse section.

Figs. 69 and 70. *C. reniforme*, (Ralfs.) Arch.  $\times 810$ . Fig. 69, front view (right-hand chloroplast of upper semi-cell in optical section); Fig. 70, optical transverse section.

Figs. 71 and 72. *C. pachydermum*, Lund.  $\times 810$ . Fig. 71, front view; Fig. 72, optical transverse section.

Figs. 73 and 74. *C. cucumis*, Corda.  $\times 810$ . Fig. 73, front view; Fig. 74, optical longitudinal section.

#### PLATE XIII.

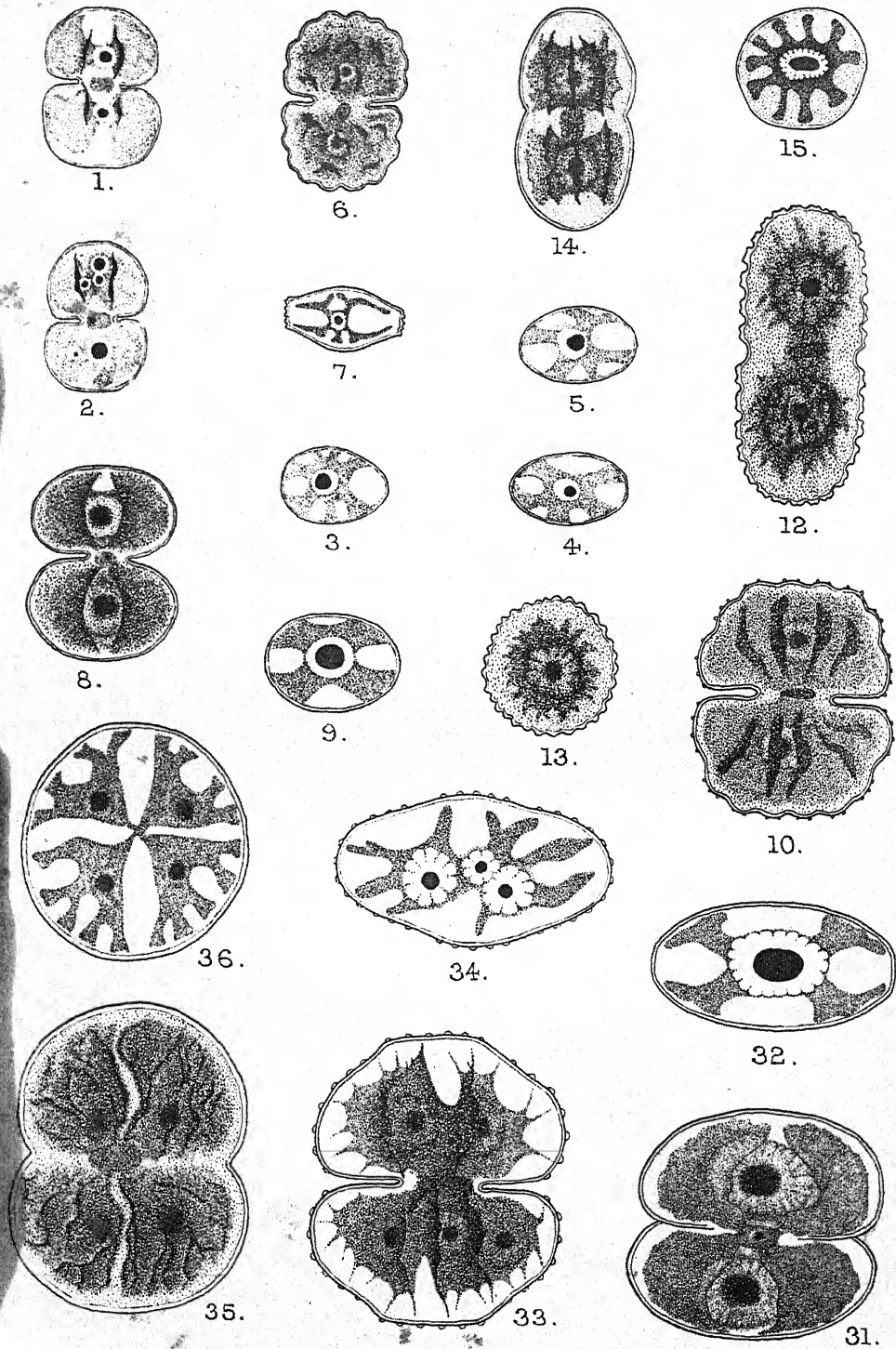
Figs. 75-81. *C. Brebissonii*, Menegh.  $\times 810$ . Fig. 75, front view; Fig. 76, longitudinal section; Figs. 77 and 78, optical transverse sections of the two semi-cells of an individual having very scanty chloroplasts; Figs. 79-81, transverse sections of various individuals.

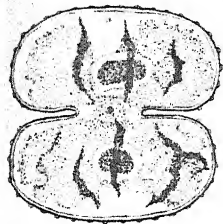
Figs. 82 and 83. *C. ovale*, Ralfs.  $\times 510$ . Fig. 82, front view; Fig. 83, optical longitudinal section.

Figs. 84 and 85. *C. Askenasyi*, Schmidle.  $\times 510$ . Fig. 84, front view; Fig. 85, optical transverse section.

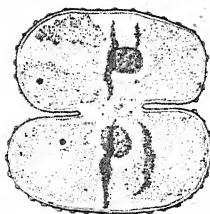
Figs. 86-8. *C. controversum*, West.  $\times 810$ . Fig. 86, front view; Fig. 87, optical transverse section; Fig. 88, optical longitudinal section.



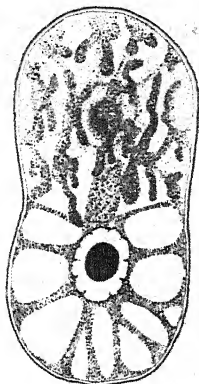




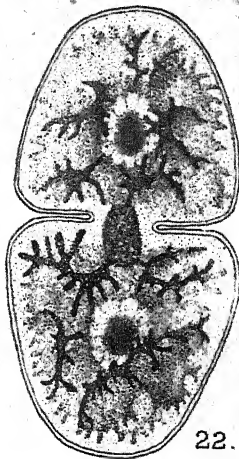
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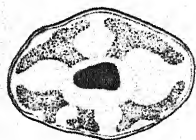
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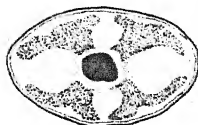
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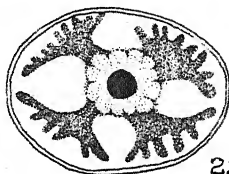
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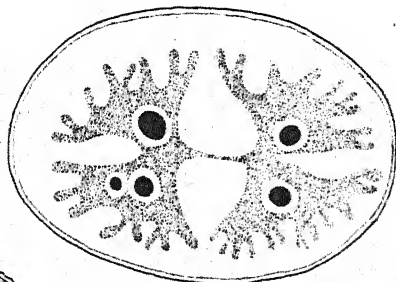
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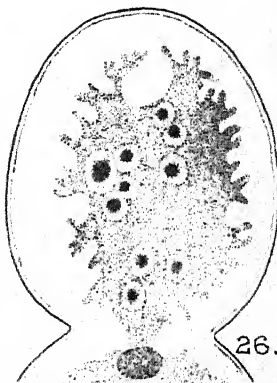
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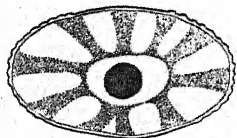
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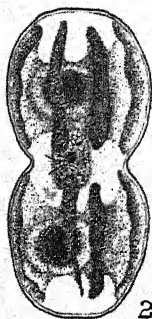
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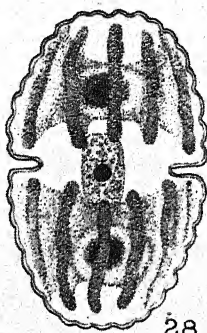
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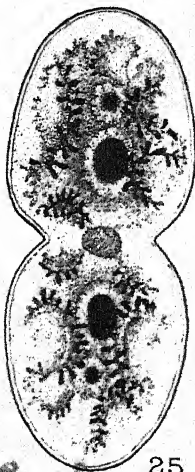
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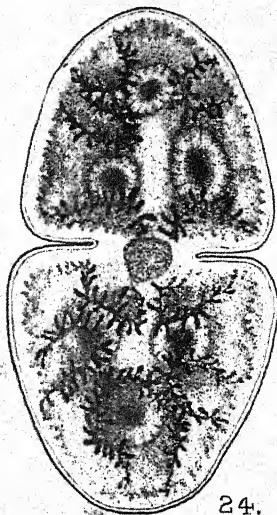
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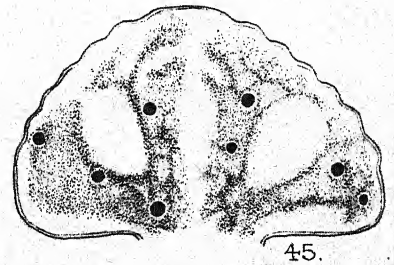
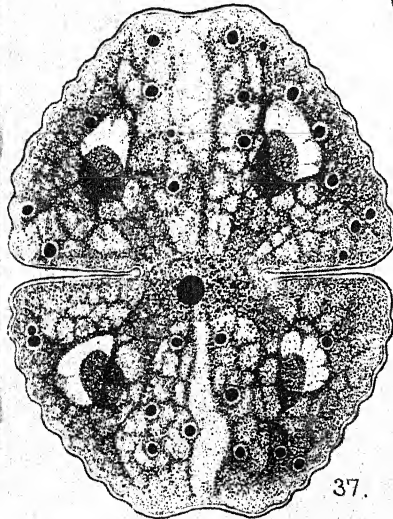
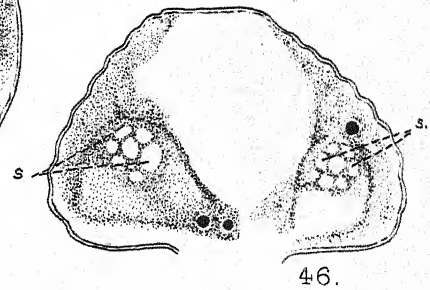
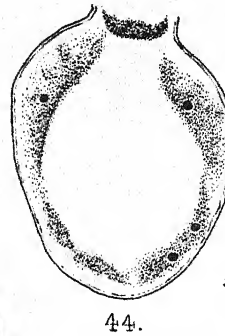
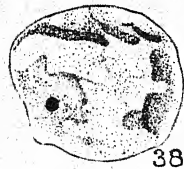
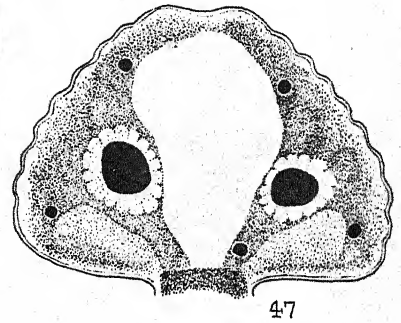
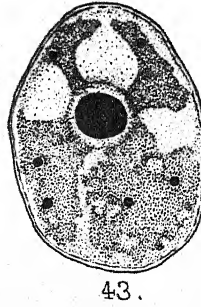
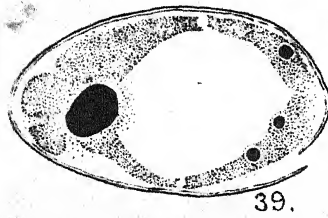
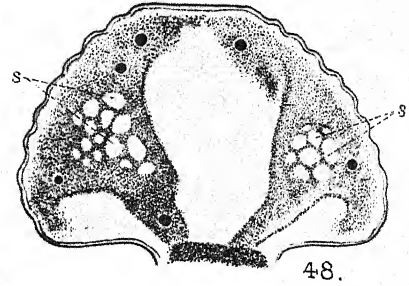
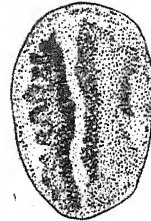
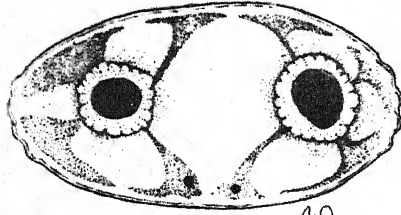
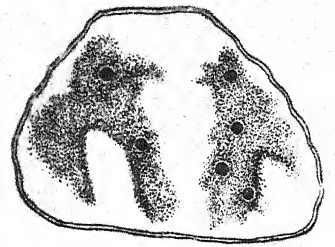
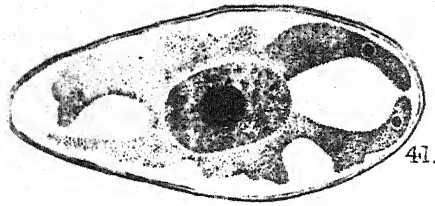


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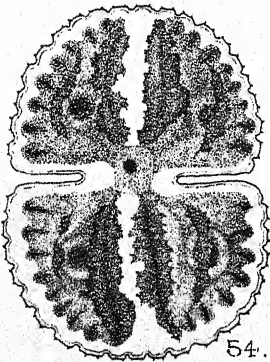
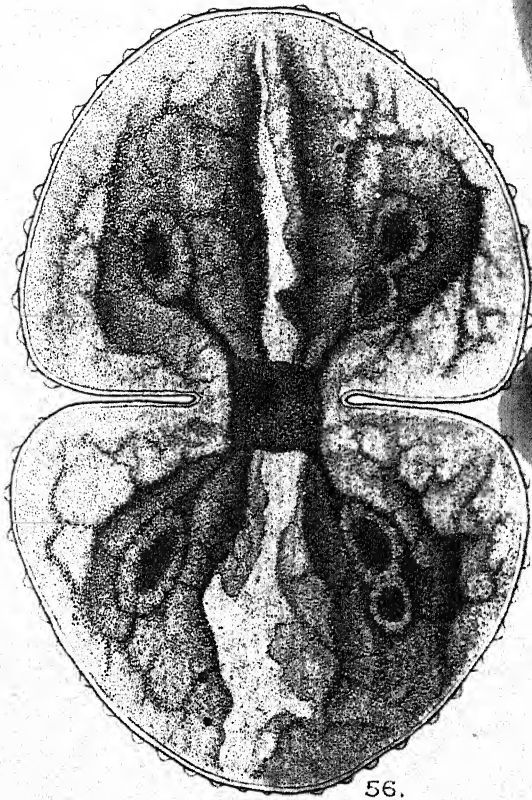
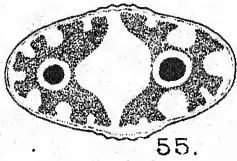
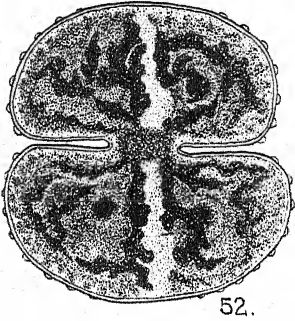
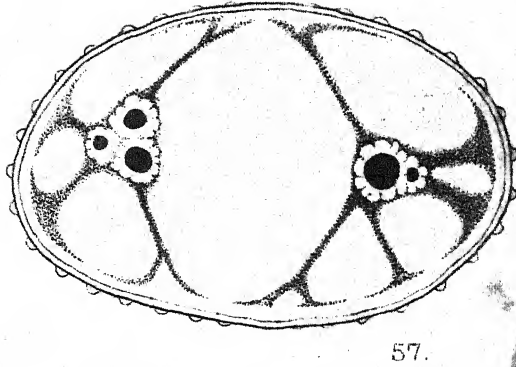
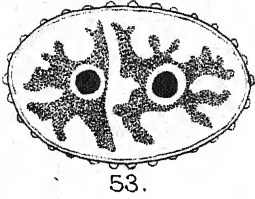
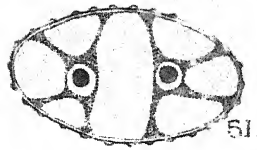
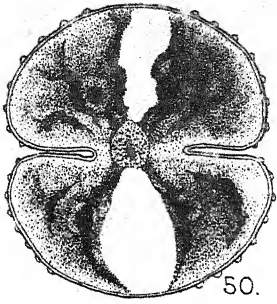


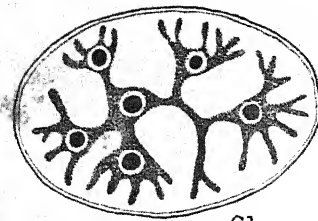
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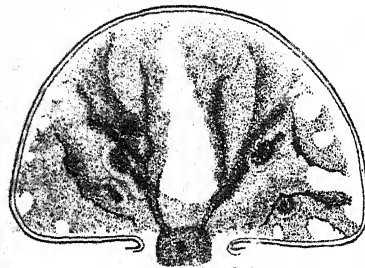




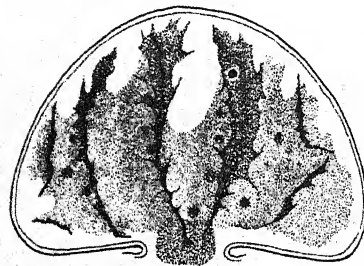




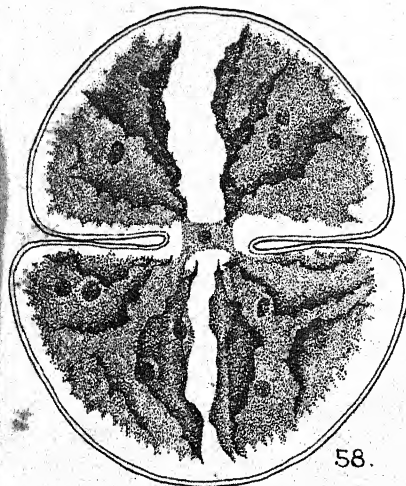
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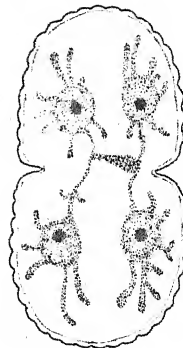
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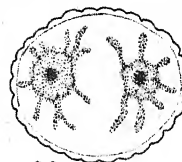
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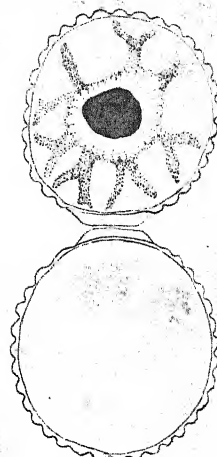
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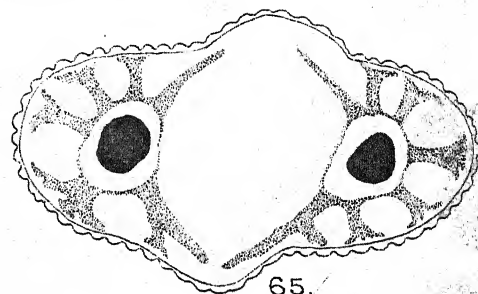
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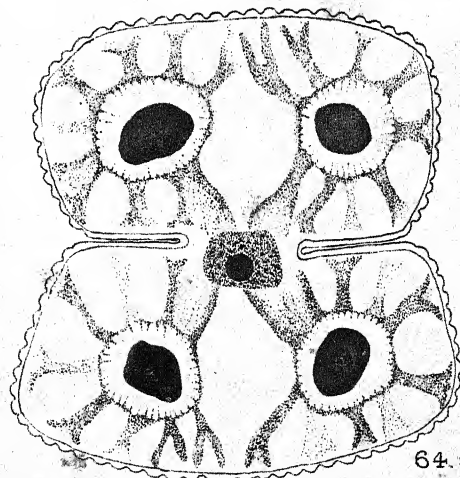
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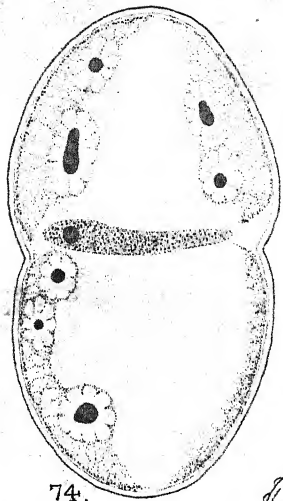


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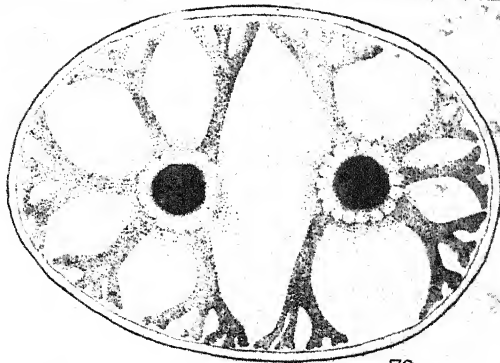


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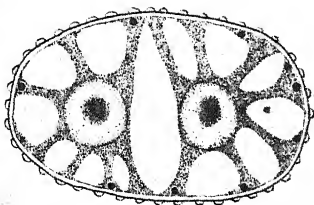
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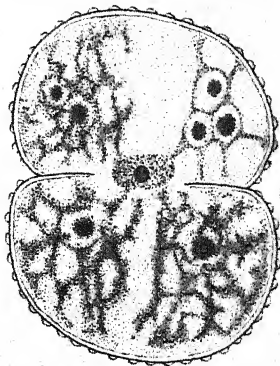
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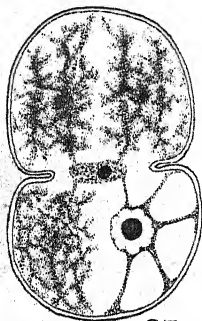
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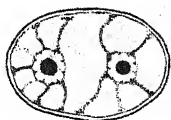
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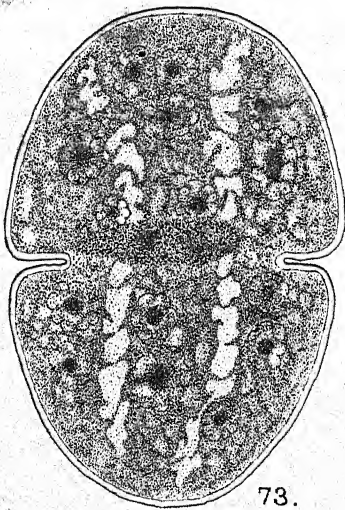
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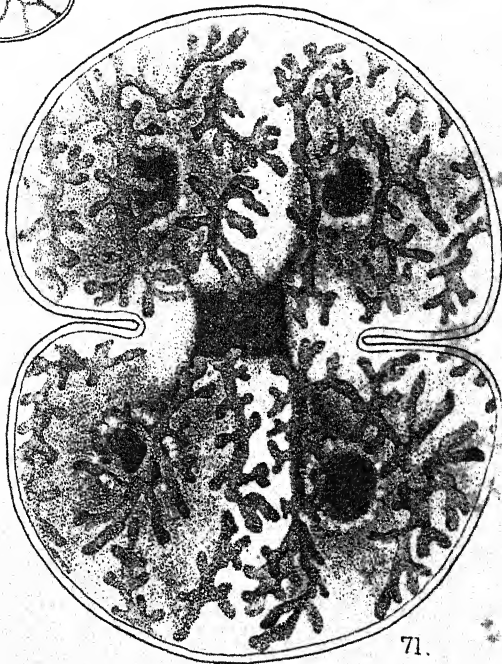
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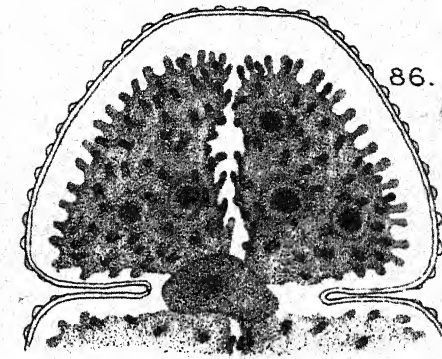
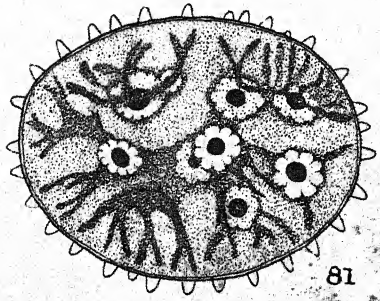
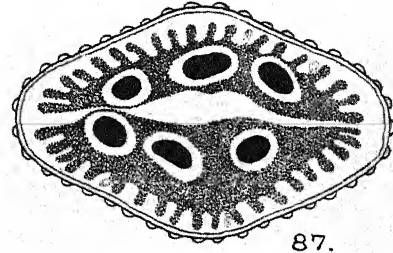
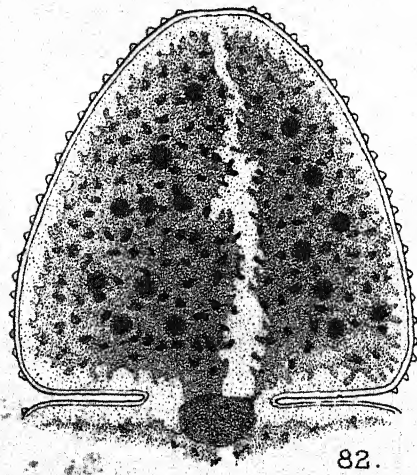
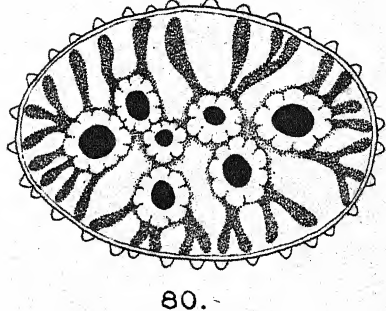
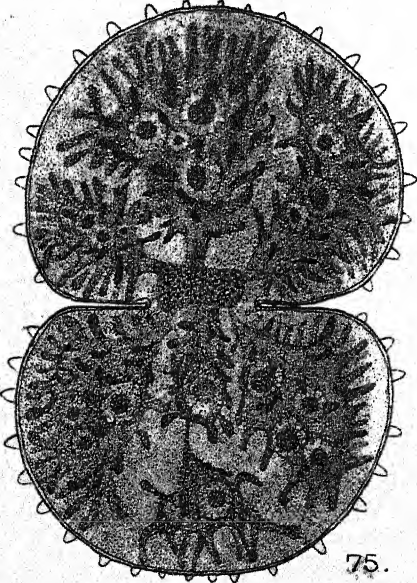
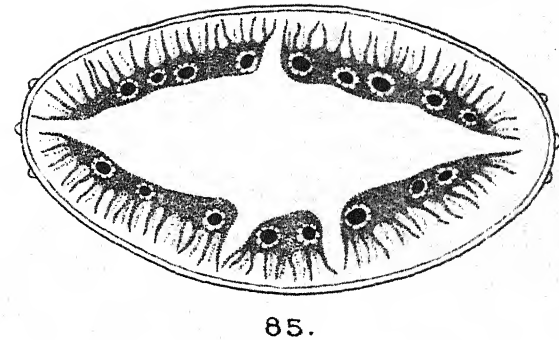
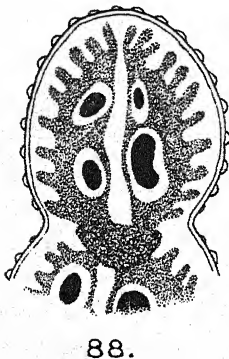
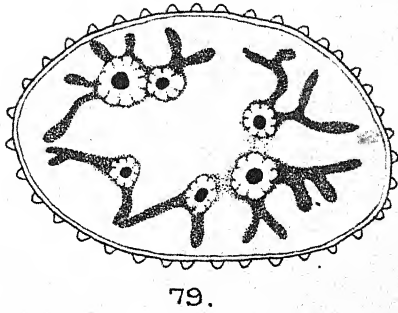
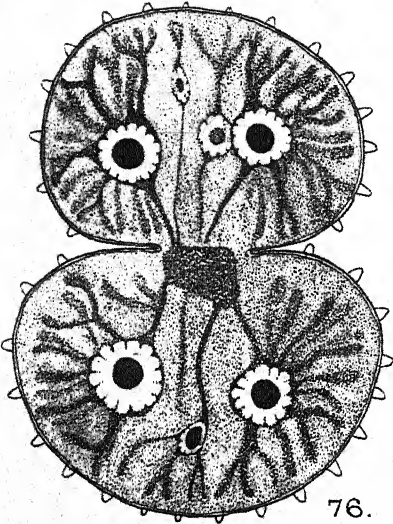
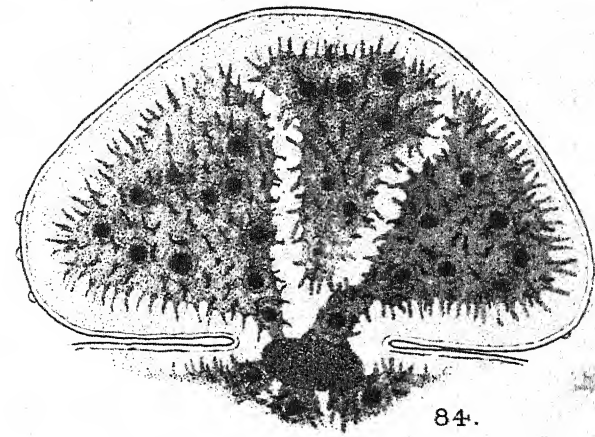
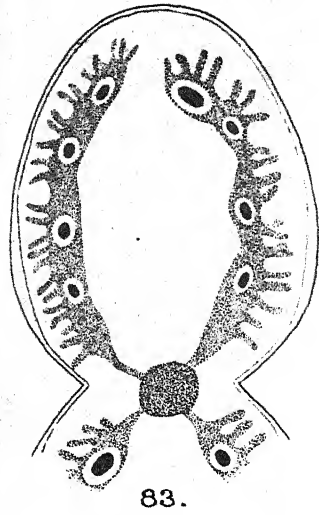
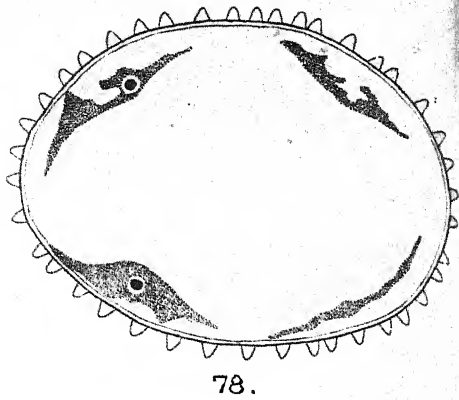
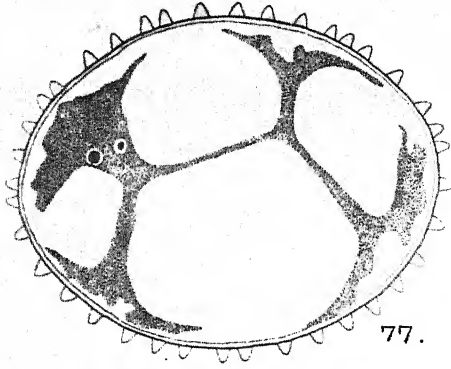


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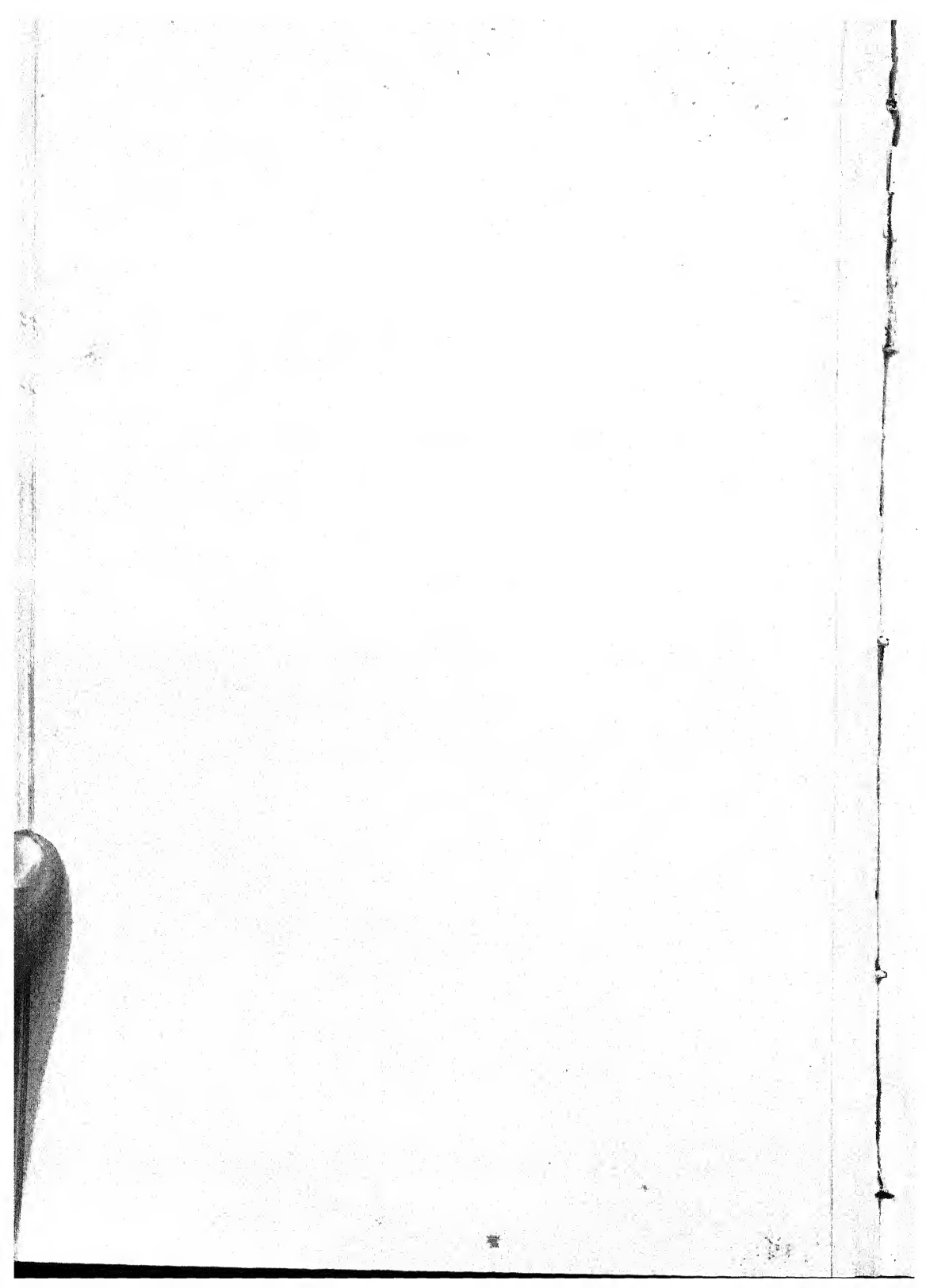


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# Plant Succession and Plant Distribution in South Africa.

BY

J. W. BEWS, M.A., D.Sc.,

*Professor of Botany in the Natal University College.*

## INTRODUCTION.

ANY study of plant distribution in any country must take into account the interesting hypothesis of age and area, which has been fully illustrated by Dr. J. C. Willis in numerous recent papers (14-21). When his first papers on Ceylon appeared, I was engaged on a fairly detailed analysis of the extensive grassland flora and plant succession in South Africa (8), and a somewhat superficial application of the age and area hypothesis to the South African grasses seemed on the whole to confirm Willis's results for Ceylon. Endemic species of Gramineae in South Africa tend to have comparatively narrow ranges, while non-endemic species have usually wider ones. At the same time, even granting these facts, and the much more definite ones brought forward by Willis, to be true (as of course they undoubtedly are), and granting further the perfectly reasonable contention that the longer a species exists in any country the wider will be its distribution in that country, yet it appeared clear that in a country like South Africa, with its great variations in climatic conditions, the action of the age and area rule must be profoundly modified.

The south-western region in South Africa is distinguished by a winter rainfall and dry summers, and has a distinctive flora with a very large number of endemic species which range all over it (5). The eastern side of the sub-continent has summer rains, and a flora which differs very greatly from the south-western. Nevertheless, in the south-western region, even in the Cape Peninsula on the western side of it, there are small outliers of eastern forest in certain moister climatic situations. The species composing such forests are eastern species, and for the south-western region would rank as 'wides', yet they have a small range in the south-western region, and would be considered as rare, or rather rare according to Willis's scheme. In the same way numerous south-western species extend eastward along

the mountain ranges to the Drakensberg (detailed lists have been given in some of my former papers (1, 3, 4)). As far as the eastern region is concerned they are 'wides', yet they have an extremely restricted range in the eastern region. It may be emphasized that these are not a few isolated exceptions, for the numbers are considerable.

Again, if we take Natal, we find that on the tropical coast-belt there are a large number of tropical species which have a wide range to the north through the tropics. Out of a total of about 1,500 species 545 are recorded also in the 'Flora of Tropical Africa', or 36.3 per cent. These extend north of the Tropic of Capricorn. Now practically all those 545 species have a very narrow range in Natal, being confined to the frost-free localities of the narrow coast-belt, yet they are all 'wides' as far as Natal or South Africa is concerned. If we attempt to deal with Natal as a whole on the same lines as those adopted by Willis for Ceylon the results obtained are utterly different. The reason of course is obvious. Natal is a country with very diverse climatic conditions. One set of 'wides' from the south-west have a restricted distribution along the mountains of Natal, another set of 'wides' from the tropics have a restricted distribution along the coast-belt of Natal.

This line of criticism has to a large extent been met by Willis, though, as he admits himself, it is unfortunate that he did not do so in his earlier papers. In his reply to criticism by Ridley (12) he states (17, p. 191), 'I much regret that in my Ceylon papers I did not make sufficiently clear the various conditions that might modify the action of my age and area rule. Partly this was because I thought that many were obvious, and partly because I was thinking more of making the law itself clear.' He goes on later to give a list of such modifying causes as follows (17, p. 206):

1. Chance (the operation of causes as yet not understood);
2. Action of man in opening up a country, cutting of forest, exploring, making fires, &c., &c.;
3. Interposition of barriers, such as mountains, broad rivers, desert, arms of the sea, sudden changes of climate from one district to the next, and the like;
4. Geological changes, especially if involving change of climate;
5. Serious changes of climate;
6. Natural selection;
7. Local adaptation;
8. Dying out of occasional old species;
9. Arrival of a species at its climatic limit;
10. Density of vegetation upon the ground at the time of arrival of a species;
11. Presence or absence of mountain chains;

12. Relative width of the union between the country of departure and that of arrival.

He states further: 'Some of these causes probably come into action in almost every single case of any individual species, though upon large numbers and in the long run they cancel out. All might have been covered had I added to my tentative statement of the law the phrase "so long as conditions remain constant", or words to that effect.'

The chief causes which apply to Natal are Nos. 3 and 9 as given above, but the numbers involved are so large that there can be no question of their cancelling out, nor does a phrase such as 'so long as conditions remain constant' cover everything. It is not a question of present conditions remaining constant so much as that the law of age and area applies only to regions where conditions are fairly uniform, as in Ceylon and New Zealand. It is only in his latest paper on 'The Flora of Stewart Island' (21) that Willis expressly recognizes this, but it appears to me to be the fact which above all others requires emphasis. In the same paper (21, p. 23) Willis draws a distinction between ecological and taxonomic distribution, and states that it is to the latter that age and area refers. 'The two lines of work', he says, 'are really very distinct, and have comparatively little overlap. The plants are locally distributed, within the area which is assigned to them by the passage of time, in accordance with their reactions to the various ecological factors which are operative there. Ecology simply seems to make what it can, so to speak, of the floras with which it is provided by the mere action of phylogenetic descent and of time.'

Whether or not the two lines of work are as distinct as Willis believes, it seems clear that ecological principles must modify the action of the age and area rule, and the object of this paper is to see how far plant distribution in South Africa can be explained on purely ecological lines. Certain general principles are put forward which, so far as I am aware, have not been expressly stated before. The very fruitful concept of plant succession is entirely ignored by Willis, yet it throws light on many facts which otherwise would remain obscure.

#### WIDELY DISTRIBUTED SPECIES IN SOUTH AFRICA.

The fact that the only general 'flora' of South Africa, the 'Flora Capensis', is still incomplete, and more than half the total number of families were dealt with in the first three volumes, which were published nearly sixty years ago, at a time when Natal, the Orange Free State, and the Transvaal were almost entirely unexplored, makes any systematic investigation into plant distribution in South Africa a matter of considerable difficulty. We have, however, two or three fairly complete check-lists, and for our present purpose it is sufficient to pick out the species that are common to Natal on the eastern side, and the Cape Peninsula on the

extreme south-west. The list for Natal is that by Medley Wood (22), and that for the Cape Peninsula by Bolus and Wolley-Dod (10). The former contains a total of nearly 3,500 species, and the latter over 2,000. There are 320 species common to both (not including ferns). Our object is to see whether ecologically these widely distributed species have anything in common, and, if so, whether such features differ from those possessed by species with a more restricted range.

Of the 320 species, 60, or 19 per cent., are ruderals, many of them introduced, and that probably at different points. At any rate, it has always been recognized that common weeds spread very rapidly. This is due partly to man's interference with natural conditions, partly to the fact that ruderal species are all good colonizers, and are spread rapidly either by the abundance of their seed production or by efficient vegetative reproduction. The class as a whole is distinguished by belonging to early stages of successions, the initial stages chiefly of various subseries, if we use Clements's system of nomenclature (11). Ruderals are killed out by taller growing species in the later stages of the succession. Annual plants (Therophytes) are common among these ruderals, and the majority of such annuals are widespread in South Africa, though other classes of annual plants are not.

Another 65 out of the 320 species are aquatic or marsh plants. There are 26 species of Cyperaceae, common to the Cape Peninsula and Natal, 7 Juncaceae, including the Palmiet (*Prionum palmita*) which forms large consocieties, 5 Naiadaceae, and many other isolated species, e.g. *Nymphaea stellata*, *Gunnera perpensa*, *Epilobium hirsutum*, *Limosella aquatica*, *Utricularia livida*, *Typha capensis*, *Phragmites communis*, *Agrostis lachnantha*, *Diplachne fusca*, *Polygonum monspeliensis*.

It has long been realized by ecologists that water and marsh plants tend to have a wide distribution, and that the floristic composition of marsh vegetation tends to vary but little, even in different climatic areas, or with increase of altitude, &c. This fact was pointed out in detailed ecological papers dealing with the vegetation of Natal (2, 4). Water presents a relatively uniform environment everywhere, but it is also a relatively unstable one. Water and marsh plants belong to the initial stages of the hydrosere, so that this large class of widely distributed plants, like the ruderals, have this feature in common, they belong to early stages of the succession. These two classes absorb between them 125 species, or 39 per cent. of the 320.

The remaining 195 species belong to the xerosere. Among them are included many Drakensberg species, e.g. *Metalasia muricata*, *Stoebe cinerea*, *Erica cerinthoides*, *E. hispidula*, species of *Helichrysum*, &c., which, as already mentioned, represent outliers of the south-western flora on the eastern mountains (though a great many such Drakensberg species which are really south-western do not extend quite so far west as the Cape

Peninsula, and are not included in the 320). On the other hand, the eastern (Natal) trees and shrubs which occur in the Cape Peninsula are confined to the small forest areas on the slopes of Table Mountain, where the rainfall is greater, and the summer heat is tempered by the south-eastern mist clouds.

A few species which are common to Natal and the Cape are chiefly sea-shore plants, and extend right round the coast, e.g. *Cryptostemma niveum*, *Dimorphotheca fruticosa*, *Osteospermum moniliferum*, *Passerina filiformis*, *Mesembrianthemum edule*, *Chenolea diffusa*, *Cynanchum obtusifolium*, *Chironia baccifera*, *Olea capensis*, *Ehrharta erecta*, *E. calycina*, *Sporobolus pungens*, *Stenotaphrum glabrum*, though several of them do extend slightly inland.

A careful examination of the whole 195 shows that, with the exception of one or two forest trees (*Podocarpus latifolia*, *Ocotea bullata*, *Curtisea faginea*), these also belong to early stages in the succession (the initial stages of the xerosere). Take the grasses for instance. No fewer than 43 species are common to the Cape and Natal. Of these, 18 are either ruderal or vlei (marsh) grasses, 4 are sea-shore species, and the remainder are all primitive colonizing types, even including the xerophytic varieties of *Anthistiria imberbis*, which are found in the Cape Peninsula. The species *Sporobolus indicus*, *Eragrostis curvula*, *Aristida angustata*, *A. barbicollis*, *Andropogon hirtus*, *Cynodon dactylon* are examples. Reference to my published work on 'The Grasses and Grasslands of South Africa' (8) will make clear how closely similar the grasslands of the whole western side of South Africa are to the initial stages of the succession in the eastern grassveld region. The chief pioneer genera which help to establish grassland in all the eastern areas are *Aristida*, *Eragrostis*, *Sporobolus*, and *Cynodon*. These same genera are dominant in the climax stages of the primitive semi-open grassveld of the drier areas towards the west, and extend through the Karroo and semi-desert formations as well.

The trees and shrubs which are common to the Cape Peninsula and Natal are also particularly interesting. Some of them, e.g. *Ilex mitis*, *Myrica conifera*, are pioneers in the hydrosere, but the majority are light-demanding, deep-rooted; xerophytic species which in Natal grow outside the close forest, and act as pioneers in the xerosere, preparing the way for the more mesophytic, more ombrophilous kinds. I have described in detail elsewhere (6, 7) how *Acacia horrida* (*A. karroo*) is the most important pioneer in the extensively developed thornveld. It invades grassland, and a great many species which cannot themselves act as pioneers, grow up underneath it and finally often overtop it and kill it. It is one of the most widely distributed species in South Africa. Another pioneer species, *Myrsine africana*, which is very important in the establish-

ment of forest in the Drakensberg (4), extends from Natal to the Cape. The most important pioneer trees and shrubs in Natal are included in the following list, which very nearly exhausts all those common to the Cape Peninsula and Natal: *Rhus villosa*, *R. pyroides*, *R. lucida*, *Cunonia capensis*, *Olinia cymosa*, *Sideroxylon inerme*, *Royena lucida*, *Celastrus buxifolius*, *C. acuminatus*, *Myrsine africana*, *Olea verrucosa*, *Celtis rhamnifolia*, *Kiggelaria africana*, *Scolopia mundii*, *Grewia occidentalis*, *Noltea africana*, *Cliffortia strobilifera*, *Psoralea pinnata*, *Plectronia ventosa*, *Halleria lucida*. *Myrsine melanophleos* is a forest tree which, though it can withstand shade, also often acts as a pioneer, being rather widely adaptable.

There are a few light-demanding climbers which extend from the Cape to Natal, e.g. *Clematis brachiata*, *Scutia indica*, *Vitis capensis*, *Asparagus africana*, *A. aethiopicus*, *A. medioloides*; and a few parasitic species have the same wide distribution, e.g. *Cassytha capensis*, *Harveya coccinea*, *H. purpurea*, *H. squamosa*, *H. bolusii*, *Melasma sessiliflorum*, *Thesium spicatum*, *Viscum obscurum*. The wide distribution of these latter is not easy to explain either on ecological lines or by the age and area rule, for presumably they are younger than their hosts.

It is interesting to find that the species which commonly take possession after fire, e.g. *Rubus pinnatus*, *Polygala myrtifolia*, *P. virgata*, are again widespread types. They represent once more the initial stages of subseres.

The result of this comparison of widespread species in South Africa appears to be that while they are seen to belong to widely different growth forms, and to show no possible phylogenetic relationship, yet with very few exceptions they agree in belonging to early stages of the plant succession. They act as pioneers which colonize either waste land or cultivated fields, or burnt-out forest, or they invade lakes and pools and vleis, or they colonize sand-dunes and open sea-shore habitats, or they belong to early stages of the main xerosere, and often play an important part in the establishing of grassland or forest plant communities. The results, then, appear to justify the putting forward of the following hypothesis:

*Species with a wide distribution are usually found in an early stage of the plant succession.*

The rule probably only applies to countries where there are great variations in climatic conditions, such as is the case in South Africa, where the climax types in drier parts so often represent the initial stages of succession in moister regions. This result is not to be considered as in any way contradictory to the age and area law already discussed. It is rather an ecological amplification of that law, and it is bound up with other principles of plant succession.

Succession as a rule proceeds from extremes, where there is either too much or too little water, towards the mesophytic, and the highest stage of



development is forest. If now we suppose that two separate groups of species were produced by mutation at the same time, one inside the forest, the other outside, but, as might easily happen, very near to each other geographically, the former would only spread up to the limits of the forest climatic area. In South Africa at the present time such areas are comparatively limited in extent, and are separated by drier areas, which any forest species has difficulty in crossing. The species produced outside the forest are of necessity of a more xerophytic light-demanding type, and are able to spread over all the drier areas, but cannot invade the forest. They can, however, extend into regions, such as all the dry western parts of South Africa, where the climatic conditions are such that no forest development is possible. In course of time they come to have a much wider distribution than the species which we have postulated to be of the same age.

It may be well to point out that it is not necessary to take a large area like the whole of South Africa to find an application for the above principle. Even in smaller areas, such as Natal, it applies equally well. The early stages of succession contain the same species on the coast-belt, in the midlands, and in the mountain regions, but later stages differ much more in the different parts. Grassland is far more uniform over Natal than the scrub and forest which invade it. The widespread species over any area, large or small, belong to early stages of the succession. It must be remembered that in Natal climatic variations are great, even in small areas, the valley climates differing from those of the surrounding hills. The climax type in the former is dry thorny scrub, but that of the latter mesophytic forest. Nevertheless the differences in the two types of grassland which precede (the low veld in the case of thorny scrub and the high veld in the case of forest) are only slight. The same grassland species range over both (2, 5).

Though it appears to be true (for South Africa at least) that widespread species appear early in the succession, it does not of course follow that all species which appear early in the succession are widespread. Many pioneer species, e.g. numerous species of *Crassula*, which colonize rocks, are, so far as is known, not particularly widespread. Such species have not reached the limits of their climatic areas, and, if we apply the age and area law to them, may be ranked as relatively young; but it should further be remembered that pioneer species are readily killed out by species which appear later in the succession, and for strict pioneers any close relatively stable plant community is itself a barrier. Most species of *Crassula* cannot invade grassland, much less forest, and they may find it difficult to migrate from one rocky region to another across great stretches of grassland.

## SPECIES WITH RESTRICTED DISTRIBUTION IN SOUTH AFRICA.

It is interesting to inquire how far the converse of the above rule (namely, that species with a restricted distribution or narrow range belong to late stages of the succession) does hold for South Africa. It is clear, from what has already been said, that it does not hold altogether, for pioneer species may have a restricted distribution. Nevertheless there are certain large classes of rare or relatively rare species (using rare in the sense used by Willis, of being restricted to a fairly small area) which, it will be shown, belong of necessity to climax or subclimax stages of succession. In a paper (now in the press) (9) dealing with the plant ecology of the Natal coast-belt, I have analysed the floristic composition of the vegetation, and have pointed out that as the succession advances in the various seres (hydrosere, psammosere, xerosere, halosere), the vegetation becomes more and more tropical, 84 per cent. of the genera and 36 per cent. of the 1,500 coast-belt species extend through the tropics, and there are in addition a large number of endemic species. All these tropical and endemic coast-belt species do not spread outside the frost-free localities on the Natal coast-belt, and as far as South Africa is concerned they are rather rare. Almost all of them appear late in the succession in scrub or forest. The marsh plants and even the grassveld species, with one or two exceptions, are all the same as occur in the midlands of Natal and other colder parts of South Africa. Among the grasses it is particularly interesting to compare the various species in the coast grassveld, which, with the exception of three or four, are the same as over the whole eastern grassveld region, with the numerous tropical or endemic species of *Panicum*, &c., which are abundant round the moist fringe of the coast forest, but do not enter into grassveld. The coast scrub and forest have been analysed in considerable detail, and over 700 species have been listed and symbols of relative frequency given. The great majority of them do not occur elsewhere in South Africa.

There are over 60 species of Acanthaceae alone, and the family is not even represented at the Cape. The numerous climbing species of Asclepiadaceae are quite distinct from the more widely distributed grassveld and western species of the same family. Tropical members of the families Anonaceae, Capparidaceae, Bixineae, Urticaceae, Euphorbiaceae, Cucurbitaceae, Convolvulaceae, Amarantaceae, Verbenaceae, Leguminosae, &c., are nearly all confined to this subclimax or climax type (scrub and forest) on the Natal coast-belt. Whether 'wides' or endemics, they are all restricted in their distribution in South Africa.

Elsewhere on the eastern side it is also true that a great many species with restricted distribution are forest species. Two endemics which are often completely dominant in forests (*Podocarpus falcata* and *Xymalos*

*monospora*) are among the number. A very large proportion of the total number of species, however, are geophytic herbaceous forms, which are scattered among the grasses of the grassveld, and next to nothing is really known about their distribution. Many of them are certainly widely distributed, e. g. those named in my work already referred to, but these also obey the general rule given above, for they are vernal plants and increase when the plant succession is sent back by grass burning or overstocking, i. e. they belong to an early stage of the succession.

Over the whole dry interior, the Karroo, the sand veld, and the western regions, the vegetation is mostly of a semi-open primitive type, and, though practically the whole of these great areas is insufficiently explored to enable any exact information to be given as to the distribution of the different species, yet many of them are at least known to be widespread, though at the same time they may be endemic.

The south-western or Cape region, where the climax stage of the plant succession, except in a few forest areas, is *Macchia*, is of peculiar interest in many ways. The number of endemic species is extraordinarily high. Some of them range all over the region, others are very rare indeed, at least as far as is known, but again much more information is necessary before any very definite statement can be made. The climatic conditions over the area are on the whole fairly uniform, and interesting results should be obtainable by applying the age and area law to this region.

So far I have confined my attention to South Africa, in this attempt to throw some further light on the question of plant distribution by applying ecological, and particularly successional, principles. At present I do not propose to enter into further details with regard to South Africa, or to attempt to apply the same methods to other countries, though there is much information scattered through ecological literature which has a bearing on the question. Thus Smith, in his Presidential Address to the British Ecological Society (13), dealt, *inter alia*, with the reasons why some grasses are widely distributed in Britain. It is interesting to find that he laid stress on their possessing high powers of surviving critical periods, especially winter and drought, but certain colonizers he notes as being restricted in distribution. Of course it is not claimed, even for South Africa, that pioneer species are necessarily widespread, though widespread species usually appear early in the succession. The climate of Britain, too, is very uniform as compared with that of any part of South Africa, and where the ecological sorting out depends more on edaphic than on climatic conditions, it is uncertain whether my rule will apply. The important thing, however, seems to be that any widespread species must have the environmental conditions which suit it also widespread. Early species in the succession, which have to be adapted chiefly to the inorganic environmental factors, are more likely to find those conditions to which they are

suited widespread over any area, than those species which belong to complex climax plant communities, where the surrounding vegetation is the most important factor for any particular species. This is especially true for the very mixed forests of Natal or any other subtropical or tropical region.

Willis, in his various papers, seldom refers to the ecological side of the question, but one or two of his statements are significant. Thus, in his Ceylon paper, he says (15, p. 13), 'The number and proportion of endemics are far greater in the wet south-western zone, i.e. in the broken hilly country of Ceylon, than in the flat and uniform dry country which surrounds it to the north-east and south-east', and of course he has already shown that endemics are relatively restricted in their distribution. The fact that they belong to the moister parts agrees very well with what happens in South Africa, but in the moister parts the highest stages of the plant succession are able to develop, and it is to these complex plant communities that the endemics and species with restricted distribution belong.

Again, in his first New Zealand paper (16), Willis's figures show that in the Cyperaceae, a family which is fairly uniform in its ecological behaviour, since most of its members belong to early stages of the (hydrosere) plant succession, both endemics and wides are widely distributed. Willis explains this by stating that the family is very old in New Zealand, but it can be equally well explained by the theory outlined above.

#### SUMMARY.

1. Willis's 'age and area' law is discussed, and in general accepted, but it is pointed out that it can only apply to regions where conditions are fairly uniform, and in South Africa, where climatic variations are extreme, its operation is greatly modified.

2. Species (320 in number) which are widely distributed over South Africa are compared, and it is found that such species usually are found in an early stage of the plant succession. They include many ruderal species which colonize waste land, &c., many aquatic and marsh plants which belong to early stages of the hydrosere, and the remainder are nearly all xerophytic, light-demanding species, which belong to early stages of the xerosere, and are often important pioneers in the establishing of grassland, scrub, or forest plant communities.

3. On the other hand, some strictly pioneer species are not widely distributed. In many cases this is probably due to the more stable plant communities acting as barriers and retarding the spread of the pioneers.

4. Certain large classes of species with a restricted distribution in South Africa are shown to belong to climax plant communities such as the coast-belt forest of Natal, as well as other forests. Reference is made to work recently completed (9) in which it is shown that in a subtropical

region such as the Natal coast-belt, as succession advances, the vegetation becomes more and more tropical, so that, while all the earlier stages of the succession in this area are widespread over the rest of Natal or farther, there are some 700 tropical or endemic species which are more or less confined to the climax or subclimax forest or scrub of the Natal coast-belt, and are thus very restricted in their distribution, so far as South Africa is concerned.

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## Radio-activity and Normal Physiological Function.

BY

V. H. BLACKMAN.

THE radio-active substances, or radio-elements as Soddy calls them, are characterized by the spontaneous and continued discharge of rays or particles which are projected from them with various speeds, sometimes with a velocity as great as that of light. The view put forward by Rutherford and Soddy in 1903, that this peculiar activity is due to a process of atomic disintegration, is now universally accepted. The rays projected are of three types— $\alpha$ -rays,  $\beta$ -rays, and  $\gamma$ -rays, some elements like radium and uranium giving off all three, while others exhibit only a single kind.

The  $\alpha$ -rays or particles are projected with great velocity, but are easily absorbed by thin sheets of metal. The  $\alpha$ -particle is now known to be a helium atom (at. wt. = 4) carrying two unit charges of positive electricity; hence the term *positive* rays is applied to them. The  $\beta$ -rays are more penetrating than the  $\alpha$  ones; they are known as *negative* rays; for they consist of negatively charged particles, in fact they are electrons. The  $\gamma$ -rays are similar to X-rays, being light waves of very short wave-length, only one-thousandth of that of visible light.

One would expect that the rays projected from the radio-elements would have a marked physiological action, and we find that they will coagulate protein, will cause 'burning' of the skin, and other deleterious physiological actions. It is also well known that radio-active substances have been used in the control of cancer, and that plants are markedly affected by exposure to these substances.

Most of the radio-active elements, like radium, actinium, and thorium, have high molecular weights and are not normally found in either animals or plants, so that their radio-active processes cannot be considered part of the normal physiology of the organism. In 1907, however, it was shown by Campbell and Wood that radio-activity is not confined to the heavy elements, but that potassium and rubidium emit  $\beta$ -rays; those of potassium having a penetrating power about equal to those of uranium, but of an intensity only about one-thousandth of that of an equal weight of that element. Now potassium is an element which is a constituent of the bodies



of all living organisms, so that the question naturally arises of the importance in normal physiological processes of the  $\beta$ -particles, or electrons, which are continually being shot off from the atoms of potassium. In 1915 H. Zwaardemaker, a Dutch worker, took up this question in relation to animal physiology and carried out a number of observations. He has recently given in English an account of his work in the 'Journal of Physiology' (vol. liii, p. 273-89, Feb. 1920). As his results are of fundamental importance for plant as well as animal physiologists, it seemed important that some brief résumé of them should appear without delay in a botanical journal.

Potassium in a quantity of about 40 grm. is found in the human body and is also an important constituent of the blood, where it occurs in the ionic state, the amount being estimated at about 1 grm. The importance of potassium for the beat of the heart is easily shown by experiments on the frog's heart. If the heart is supplied with Ringer's solution (NaCl 0.67 per cent.,  $\text{NaHCO}_3$  0.02 per cent.,  $\text{CaCl}_2$  0.02 per cent., KCl 0.01 per cent.) it will beat for hours; if the potassium is omitted from the solution the pulsation ceases in about 30 minutes. Zwaardemaker took advantage of this well-known fact to obtain information as to how far the action of the potassium ion is to be referred to its radio-active power.

It was shown many years ago that rubidium could replace potassium in Ringer's fluid, but these two elements are not only both radio-active but chemically very similar, so that such a result throws no light on the problem in question. If, however, potassium could be replaced by a radio-active element of entirely different chemical nature, such as one of the heavy radio-elements, the physiological importance of radio-activity would be demonstrated. Zwaardemaker therefore set himself to determine if substances like radium, uranium, and thorium could replace potassium in Ringer's fluid. One difficulty is that of the concentration of the substance that is to be used, for, clearly, what is required is not equivalent molecular, or ionic, concentration, but what Zwaardemaker calls 'aequiradio-activity'. Rutherford has determined the intensity of the physical radio-active effects of the various radio-elements, and from these data the required strength of the substance to be used was estimated. Potassium, as already stated, emits  $\beta$ -rays of only one-thousandth the activity of the  $\beta$ -rays of the same quantity of uranium; radium again is  $10^{-9}$  times as active in this respect as potassium.

From such data Zwaardemaker calculated the amount of radio-element to be added to the Ringer's fluid to expose the heart-muscle to the same intensity of radiation by  $\beta$ -rays as when the normal potassium is used. Armed with this knowledge, Zwaardemaker in 1917 succeeded in demonstrating that uranium, thorium, and radium, when added in aequiradio-active quantity, can replace potassium in Ringer's fluid, not only enabling the heart to continue beating, but restoring the pulsation of a heart which has

stopped from the absence of potassium. Later, ionium was added to the list, and also lanthanum and cerium, which, owing to contamination with actinium, appeared to possess a trace of radio-activity. The gaseous emanation (Ramsay's niton) which escapes from radium when it is kept in aqueous solution was also investigated. This element, which emits  $\alpha$ -particles, was also found to restore pulsation to a heart which has been brought to a standstill through absence of potassium. Attempts were made to replace potassium by some non-radio-active element, but all attempts were a failure except in the case of caesium, to which, however, radio-activity has been repeatedly ascribed.<sup>1</sup>

These results were sufficiently striking, but the question naturally arose as to how far the disintegrating atom was concerned in the process; i.e. whether the corpuscular radiation alone could excite or restore the heart's function. This question was answered in the affirmative by the still more striking experiment of bringing a glass bulb, containing mesothorium giving off  $\beta$ -rays, near a heart which had been rendered motionless by perfusion with potassium-free Ringer's fluid. The pulsation was found to return invariably, sometimes in as short a time as three minutes. It was also proved later that the  $\alpha$ -radiation from polonium was in some cases competent to restore pulsation to a heart.

It will be noticed that the potassium emits  $\beta$ -radiation (negative), while the heavy elements which can replace it emit to a large extent  $\alpha$ -radiation (positive). The heart can thus be stimulated equally well by substances emitting either the negative or the positive rays. It was also observed that when substances emitting both  $\alpha$ - and  $\beta$ -radiation are supplied to the heart together, they have an antagonistic action, and in appropriate concentrations can be made to neutralize one another.

The capacity of the heavy radio-elements to replace potassium and the corresponding antagonism has also been observed recently by other workers in connexion with the retention of sugar by the kidney and in a certain number of other physiological processes.

The mode of action of these corpuscular radiations is not clear. The charged particles as they shoot along will act by induction, detaching everywhere electrons from their atoms; they also transfer kinetic energy, and when they come to rest, on, say, some colloidal complex of the cell, they will transfer their electric charge and so may set free some ion absorbed on the surface.<sup>2</sup> Whatever the nature of the action, Zwaardemaker concludes that 'radio-activity is a mighty biological factor capable of restoring a lost function'.

<sup>1</sup> It is possible that we have in the frog's heart a physiological test of radio-activity even more sensitive than the physical methods at present available.

<sup>2</sup> The radiation of the fixed potassium of the heart muscle appears to be inactive; the reason for this is not clear.

In view of the fact that potassium is an essential constituent of the plant, these results are clearly of great importance to botanists; it may be that the importance of potassium in the life of the plant may in part be explained by its radio-active power. In the minuteness of the dose of the heavy radio-active elements required compared with that of potassium, and in the ease with which a toxic concentration is reached, may be the explanation of the conflicting results obtained by the addition of radio-active earths to soil. In any case, however, Zwaardemaker's result would in no way suggest the superiority of such earths over simple potassium fertilizers.

Physiologists will welcome the deeper analysis of biological processes which Zwaardemaker has achieved, yet it must be admitted that they increase still further the difficulty of envisaging his subject which faces the worker in the field of either animal or plant physiology. To the equipment of ordinary physics and chemistry which such a worker should bear has been added of late years a knowledge of many aspects of physical chemistry, of colloid chemistry, and of some of the more special branches of electrical science; to this heavy burden must now be added a knowledge of radio-active phenomena.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,  
LONDON, S.W. 7.

# Studies on the Chloroplasts of Desmids. IV.

BY

NELLIE CARTER, D.Sc.

With Plates XIV-XVI.

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### XI. THE CHLOROPLASTS OF *STAURASTRUM*.

THE chloroplasts of some of the smaller species of this genus are amongst the earliest and best known of all Desmids, since in so many cases their structure is quite clear from the examination of the living individual in the end view. Thus Ralfs (1848) figured the chloroplasts of *Staurostrum furcigerum* and others, whilst Nägeli (1849) and Delponte (1873) also illustrated the form of chloroplast in several species. The chloroplasts were also figured in several species by W. and G. S. West (1904-11), but in the case of the larger species of the genus the disposition of the chloroplasts is more obscure, and in many cases their structure is not known. It has often been assumed that these large species with very densely green cell-contents were provided with parietal chloroplasts, and Lundell (1871) separated several such species and placed them in a new sub-genus *Pleurenterium*, this being raised by Lagerheim (1888) to the rank of a genus. As in the case of *Pleurotaeniopsis*, however, such a division is undesirable, as explained by W. and G. S. West (1904-11), particularly in view of the fact that many of the species included by Lundell in *Pleurenterium* really possess axile chloroplasts just as the majority of species of *Staurostrum*. The only species of the genus found during this work to be provided invariably with parietal chloroplasts was *St. tumidum*.

The species examined included *St. aversum*, Lund.; *St. hirsutum*, (Ehrenb.) Ralfs; *St. orbiculare*, Ralfs; *St. punctulatum*, Bréb.; *St. aculeatum*, (Ehrenb.) Menegh.; *St. pyramidatum*, West; *St. Simonyi*, Heimerl.; *St. forficulatum*, Lund.; *St. Sebaldi*, Reinsch.; *St. Mansfeldtii*, Delp.; *St. Cerastes*, Lund.; *St. gracile*, Ralfs; *St. margaritaceum*, (Ehrenb.) Menegh.; *St. paradoxum*, Meyen, var. *longipes*, Nordst.; *St. jaculiferum*, West; *St. furcigerum*, Bréb.; *St. Ophiura*, Lund.; *St. Arctiscon*, (Ehrenb.) Lund.;

*St. anatinum*, Cook and Wills; *St. sexangulare*, (Bulnh.) Lund.; *St. Brasiliense*, Nordst., var. *Lundellii*, W. and G. S. West; *St. grande*, Bulnh.; and *St. tumidum*, Bréb.

The first sixteen species agree with each other fairly well in having a simple axile chloroplast with a centrally placed pyrenoid such as was figured by Nägeli (1849). In all the remaining species with the exception of *St. tumidum*, the general plan of the chloroplast is quite similar to that of the first large group, but there are variations in the number and arrangement of the pyrenoids, and in the number of prolongations of the chloroplast running into each angle of the cell. *St. tumidum* has chloroplasts of quite different structure.

In the simplest type of chloroplast there is a fairly massive central axile mass containing typically a single pyrenoid, and from this a more or less definite lobe arises opposite each angle of the cell (Fig. 18). Each of these lobes forks sooner or later, so that there are two distinct masses in each angle of the cell. Very often this forking is so deep that there are apparently two masses or plates arising directly from the axis of the chloroplast opposite each angle (Figs. 2, 4, 6, 8, 10, 12, &c.).

The form of the plates often depends to some extent on the cell-wall, and if this is drawn out at the angles of the cell to form hollow processes or arms, then at corresponding points the plates of the chloroplasts are drawn out to form projections, often of considerable length, which are lodged in them (Figs. 7, 8, 24, 25, 34, and 35). Sometimes the lobes of the chloroplast in the angles of the cell are very thin and plate-like, in which case it not infrequently happens that each lobe forks near the periphery (Fig. 37), or that other smaller plates are given off from the axile mass towards the faces of the cell between the angles (Figs. 4 and 32). In other cases the chloroplast masses in the angles are so large that together they nearly fill the whole semi-cell, and in such chloroplasts the surface of the lobes is often ridged longitudinally, two such ridges being visible in the middle of each face of the semi-cell (Figs. 24, 25, 34, and 35). Such differences in the relative mass of the chloroplast are to be correlated with variations in the amount of stroma starch present in it.

Practically all the smaller species of *Staurostrum* examined have chloroplasts of this type, both those in which the angles of the cell are drawn out to form hollow processes, and those in which they are not.

#### *S. Sebaldi.*

Two varieties of this species were examined. The smaller, var. *ornatum*, Nordst., showed no deviations whatever from the simple chloroplast described above (Figs. 24 and 25). The larger one, however, var. *altum*, (Boldt) West, exhibited some remarkable variations in the number, size, and position of its pyrenoids. Unfortunately, it was only

possible to examine three specimens of the larger variety, and all these three differed in the arrangement and number of their pyrenoids, although the chloroplast in each case was of the ordinary type, being axile, with a pair of lobes running into each of the three angles of the semi-cell (Figs. 21 and 23). In practically every semi-cell there was also a single pyrenoid in the centre of the chloroplast (Fig. 21, lower semi-cell); in one, however, there was a group of three (Fig. 21, upper semi-cell), but that is not unusual. In all three specimens there were, however, in addition, other pyrenoids in one or more angles of the cell. Thus one specimen was quite normal in one semi-cell, but the other, in addition to a central group of three pyrenoids, had also a very small pyrenoid in one of its angles (Fig. 21, only two of the central group of three pyrenoids are visible). In the second specimen also the chloroplast was quite normal in one semi-cell, but in the other, besides the large central pyrenoid, there were also one or two very small pyrenoids in each angle of the semi-cell (Fig. 22). The third specimen also had one or two extra pyrenoids in each angle of the cell, but in this case the extra pyrenoids were of considerable size, being nearly as large as the central pyrenoids of the same cell (Fig. 23).

It may be that in the larger variety, as in var. *ornatum*, there is normally a single central pyrenoid in each semi-cell, but that, in the larger cells of var. *altum*, it is impossible in most individuals for all the accumulated products of photosynthesis to be stored in a single pyrenoid, because a pyrenoid cannot increase in size indefinitely. Therefore the original central pyrenoid may in some cases divide to produce a little group of pyrenoids in the middle of the semi-cell, or else new pyrenoids arise in the comparatively large masses of chloroplast in the angles of the cell. It seems natural that new centres of food-storage should be formed at these points, because the lobes of the chloroplast in the angles are in some cases very massive, and photosynthesis must be going on fairly extensively, whilst at the same time it would be a considerable distance for all food reserves to be transferred to the pyrenoid in the centre of the cell. The new pyrenoids are very small at first, increasing in size as stores of food accumulate until they are nearly as large as the older established central pyrenoid.

In the smaller cells of var. *ornatum* the single pyrenoid in the middle of the cell is quite sufficient for the storage of all food reserves, and the lobes of the chloroplast in the angles are neither very large, nor are they removed at any great length from the central pyrenoid, so that the formation of extra pyrenoids within them is unnecessary.

#### *St. furcigerum.*

In the form of *St. furcigerum* examined, the cell, which is triangular in the end view, is provided with two short hollow processes at each angle of the semi-cell, one above the other. The form of the chloroplast is, how-

ever, quite similar to that of the ordinary small species of *Staurostrum*. There is an axile mass of chloroplast in the centre of the cell containing typically one pyrenoid, and a biforked lobe running into each angle of the semi-cell. Each plate or lobe radiating from the central axile mass is shaped so as to project slightly into both the short processes of that particular angle.

*St. Ophiura.*

There is not much difference between the chloroplast of this species and that found in so many of the smaller species. In the latter, the lobes of the chloroplast given off from the central axile mass arise immediately opposite the angles of the cell into which they stretch, each one forking to form two plates, the two plates in any angle having been formed, therefore, from one single lobe (Fig. 8). In *St. Ophiura*, however, the lobes of the chloroplast do not arise opposite the arms of the cell, but between them (Figs. 26 and 27). The semi-cell is provided with a whorl of about eight hollow processes near its apex, and at the bases of these arms the large lobes of the chloroplast corresponding in number to the arms of the cell are drawn out at the apical region of each lateral edge to form a series of long attenuated projections which number twice as many as the massive lobes of the chloroplast given off from the central axis (Figs. 26 and 27). The projections enter the nearest hollow process of the cell-wall, and each arm, therefore, contains two projections, but these two have not been produced from one lobe of the chloroplast, but from two adjacent lobes (Fig. 27).

The chloroplast is very regular in shape, and constant in its form, showing practically no variation other than the occasional occurrence of more than one pyrenoid in the middle of the cell.

*St. Arctiscon.*

The chloroplast in this species differs from the ordinary form in that only one projection is given off from the axile mass into each arm of the semi-cell instead of two (Fig. 29). Each half-cell is provided with fifteen hollow processes which are arranged in a definite way, and the form of the chloroplast naturally depends to some extent on this. The fifteen arms are arranged in two whorls, a whorl of six arms round the apex of the semi-cell, and a larger whorl of nine round the broadest part, lower down. The large central axile mass of chloroplast contains a single pyrenoid, and gives off a single projection to each of the arms, but the projections running into the two whorls seem to be related in a particular way. They broaden out from the arms towards the axis, and certain projections filling particular arms of the lower whorl seem to be connected with corresponding projections running into closely associated arms of the upper whorl, as if at these



points a single chloroplast plate given off from the axis forked into two horizontally, one part supplying an arm of the upper whorl, and the other a corresponding arm of the lower whorl (Fig. 28, *a, a'*; *b, b'*; *d, d'*; *e, e'*, &c.). But as there are nine arms in the lower whorl and only six in the upper, it is obvious that the arms cannot be associated in pairs, one belonging to each whorl, all round the semi-cell, and on closely examining the chloroplast it is found that there are two distinct kinds of plates given off from the central axis. The larger plates stretch nearly from end to end of the chloroplast and are drawn out at their upper and lower extremities to form two prolongations which enter corresponding arms of the upper and lower whorls (*a, a'*; *b, b'*; *d, d'*, &c.). There are also shorter ridges, mere projections which enter certain arms of the lower whorl only, and do not extend as far up as the upper whorl (*c, f, i*, &c.). Altogether there are six larger plates and three smaller ones in each semi-cell, and they alternate regularly, two of the former and one of the latter all round the semi-cell.

*St. anatinum* and *St. sexangulare*.

Here the chloroplasts differ considerably from those of the other species examined in the relative size of the axis and the plates which radiate into the angles. The central axis is very much reduced, and by far the greater part of the chloroplast is to be found in a more peripheral position in the angles of the cell. Consequently the pyrenoids are not, as before, in the centre of the cell, but in the much larger masses of chloroplast in the angles (Figs. 20 and 44).

*St. anatinum* is triangular in end view, and there are consequently three masses of chloroplast, one in each angle, which towards the centre of the cell become abruptly very much thinner, being all united by a very narrow surface in the middle of the cell. Towards the periphery each mass forks to form two projections which do not extend very far into the hollow processes of the cell-wall (Fig. 20). There are typically three pyrenoids in each semi-cell, one in each angle, but frequently one or more of them divide to form a little group in well-nourished cells.

The chloroplast of *St. sexangulare* corresponds in all important respects to that of *St. anatinum*. In the material examined each semi-cell was provided with ten hollow processes arranged in two whorls of five, and the cells in end view were pentagonal, corresponding arms of the two whorls being on top of each other (Fig. 44). As before, the greater part of the chloroplast is situated in the angles, and not in the centre of the cell, and there are accordingly five masses of chloroplast, one in each angle, and each containing a pyrenoid. All five masses are connected up in the middle of the cell, and towards the periphery each one forks to form two plates, which stretch out towards the angles (Fig. 44). But in this case there are two hollow processes at each angle, and so the two plates in each angle of the

semi-cell are drawn out at their upper and lower extremities to form four projections, the two upper entering the upper arm of the angle, and the two lower the lower arm (Figs. 43 and 44).

*St. Brasiliense*, var. *Lundellii*, and *St. grande*.

The chloroplast of the large species *St. Brasiliense*, var. *Lundellii*, differs in several ways from the ordinary type of chloroplast of the genus. The semi-cells are large, and are pentagonal in the end view, and there is a central axis of considerable size in the interior which gives rise to five much larger masses, one in each angle of the semi-cell. Each of these splits up towards the periphery to form four or five distinct though narrow lamellae, whose undulating margins lie against bounding walls of the angles (Fig. 45). The pyrenoids are extremely numerous, and are scattered throughout the central axis and the larger masses in the angles, occurring in six more or less distinct longitudinal rows (Figs. 45 and 46).

*St. grande* often has a chloroplast which is in some respects very similar to that of *St. Brasiliense*, var. *Lundellii*. The cell is triangular in end view, and there is a central axis in the middle of the semi-cell which gives rise to three fairly large masses of chloroplast, one in each angle. Each of these forks into two, but the resulting plates often cling closely to the walls of the angle after the manner of parietal chloroplasts (Fig. 42). The pyrenoids are probably in typical cases seven in each semi-cell, one in the central axis, and one in each of the plates in the angles. They are usually much more numerous, however, four or five being crowded together in a row in the middle of the cell, and as many as six or seven scattered in the plates of each angle (Figs. 41 and 42).

The chloroplast of this species shows a decided tendency to vary in its disposition, and not infrequently specimens in which the interior of the cell is quite free from chlorophyll-bearing substance are to be observed, the chloroplasts being confined to the angles (Fig. 39). In such cases the central axis of the chloroplast has entirely disappeared, the masses in the angles being thus isolated (Fig. 40). Sometimes these masses in the angles retain their form as single masses forking towards the periphery, but in other cases the angle may contain two distinct plate-like structures, which embrace the walls and are practically parietal bands (Fig. 39). In these chloroplasts the pyrenoids are often two in each angle, one on each side, but they are frequently much more numerous. Sometimes the chloroplast masses in the angles are very irregular in form, and contain numerous scattered pyrenoids (Fig. 40).

Thus when truly axile, the chloroplast of *St. grande* agrees with that of *St. Brasiliense*, var. *Lundellii*, in its massive form, and in the arrangement and frequently great number of its pyrenoids.

In the case of specimens showing parietal chloroplasts it is possible that the change in form occurred in the first place in young semi-cells during cell-divisions. For in *Staurastrum* it often happens that during cell-division the more peripheral lobes of the chloroplast bud into the young semi-cell from the old one much more quickly than the central axile part, streaming up over the quickly growing cell-wall and covering it in a parietal manner, as if the mantling of the whole surface of the cell-wall with photosynthetic material were the most important thing to get completed, and as long as this were accomplished the growth of the more central part of the chloroplast would be of secondary importance. Thus even in species with centrally placed pyrenoids it is frequently seen in young though quite full-sized semi-cells that the real axis of the chloroplast is exceedingly short, and that the plates radiating from this stretch up towards the apex and angles of the cell, almost completely enclosing a large colourless space in the upper region of the semi-cell. This is even more pronounced in *St. anatinum*, where the central axis contains no pyrenoids and is consequently, in any case, not of such vital importance to the young semi-cell. In young semi-cells of this species the central axis in some cases does not exist, and even in some fully grown semi-cells, after the division of the chloroplast at the isthmus between the old and the young semi-cells, it is only represented by an exceedingly short length near the nucleus, the radiating plates arising up from this and arching over the cell-wall of the angles in a parietal manner very suggestive of the parietal chloroplasts often observed in certain cells of *St. grande*. It seems possible that, in the very short semi-cells of *St. grande* particularly, the division of the chloroplast at the isthmus after cell-division may occasionally occur actually before the axis of the chloroplast has entered the new semi-cell at all, while yet the more peripheral parts of the chloroplast have already streamed in from the old semi-cell to the new, and well covered the young cell-wall. The shape of the semi-cell, with its bulging angles and extreme shortness, would seem to be very conducive to this, for it is quite possible that the peripheral parts of the chloroplast, whilst creeping up round the cell-wall of the angles of the young semi-cell, might be cut off from the more central part of the old chloroplast by the sudden division of the chloroplast at the isthmus. This idea is supported by the fact that in some individuals the chloroplasts in one semi-cell are parietal, whilst in the other there is only one chloroplast which is axile.

This strong tendency to variation in the disposition of the chloroplasts during cell-division makes it absolutely impossible for any reliable system of classification to be based on the characters of the chromatophore, such as was attempted by Lundell and Lagerheim.

*St. tumidum.*

This species differs from all the other species of the genus examined in having chloroplasts which are probably invariable in their parietal disposition. There are usually about twelve to fifteen or sometimes more chloroplasts in each semi-cell, and they are in the form of rather narrow bands, running longitudinally, each with two or three pyrenoids embedded in it (Fig. 48). Very often the bands are fairly even in width throughout their length, their outlines are often slightly irregular, but their structure does not seem to be complicated by the presence of projections from the edges and surface, as in the case of the parietal chloroplasts of some other genera. In other specimens the chloroplasts are drawn out at certain points throughout their length to form very thin strings between the large globular masses which alternate with them and contain the pyrenoids, whilst in other cells the bands are irregular in form and do not extend from end to end of the semi-cell (Fig. 47).

## XII. THE BEHAVIOUR OF THE CHLOROPLASTS DURING CELL-DIVISION.

The general external appearance of cell-division in Desmids was first figured by Ehrenberg (1838) in *Cosmarium*, and later in other genera as well by Focke (1847), Ralfs (1848), Nägeli (1849), de Bary (1858), and Delponte (1873). In most cases, however, these investigators did not attempt to illustrate the origin of the chloroplast which eventually becomes apparent in the new semi-cell, and it is only in the figures of Focke (1847) for *Micrasterias*, and de Bary (1858) for *Cosmarium Botrytis*, that any clue is given to the behaviour of the chloroplast during cell-division. Amongst unconstricted Desmids the details of cell-division, including the division of the chloroplast, have been thoroughly investigated in *Closterium* by Fischer (1883) and Lutman (1911), and thus, although our knowledge of the processes accompanying cell-division in the Saccodermæ is fairly complete, in the much larger group of constricted Desmids nothing has been discovered since the time of de Bary, and there is no definite information concerning the division of the chloroplasts in these forms, with the exception of the very slightly constricted genus *Hyalotheca*, in which cell-division has been recently studied by Acton (1916).

In *Hyalotheca* and *Closterium*, the only two genera which have at present been investigated, the process of cell-division is not identical. In *Closterium*, as described by Lutman, the first intimation that cell-division is about to take place is seen in the chloroplasts, which show a pinching-in about one-third the distance from nucleus to apex. Several hours later the nucleus divides, and eventually a transverse wall is laid down between the daughter nuclei. The latter then become amoeboid and begin to move away in opposite directions, travelling towards the constrictions in the

chromatophores. Each nucleus finally takes up its position in the constriction, and the chloroplast then completes its division, the nucleus slipping in between the two newly-formed halves.

In *Hyalotheca* according to Acton (1916) it is the nucleus which divides first, and the transverse wall is then formed. As in *Closterium* the daughter nuclei then become amoeboid, and each one takes up a lateral position opposite the central pyrenoid of one of the chloroplasts. Under the influence of the nucleus the chloroplast and pyrenoid now divide, the nucleus slipping in between the two halves of the chloroplast as they separate.

The process in *Hyalotheca* differs from that in *Closterium* in that the chloroplast does not divide until after nuclear division has been completed, whereas in *Closterium* the chromatophores prepare for their division long before there are any other visible signs of cell-division. On the other hand the two genera agree in the amoeboid movements of the daughter nuclei.

During the present investigation the process of cell-division was studied in living examples of several species, including *Micrasterias rotata*, (Grev.) Ralfs; *M. denticulata*, Bréb.; *Cosmarium punctulatum*, Bréb.; *C. subtumidum*, Nordst.; *Euastrum Didelta*, (Turp.) Ralfs; *Eu. ansatum*, Ralfs; and *Staurastrum punctulatum*, Bréb., whilst the division process was investigated from stained preparations at various stages in several other species.

In *Netrium* and *Cylindrocystis* cell-division probably closely resembles that of *Closterium*. In Fig. 51 the first signs of cell-division are seen in *Netrium oblongum*, (de Bary) Lütke., var. *cylindricum*, West, in the constriction of the chloroplast and pyrenoid about half-way between the nucleus and apex. (In *Closterium* this constriction occurs about one-third the distance from nucleus to apex, because the apices of the cell are usually so very attenuated, but in both cases the chlorophyll-bearing material is approximately halved.) In Fig. 53 (*Netrium Digitus*) the transverse wall has been formed, and the daughter nuclei have begun to migrate towards the gaps between the chloroplasts, the latter having apparently quite completed their division without the very close association of the nuclei. Fig. 52 shows the completed division.

A rather late division stage in *Cylindrocystis crassa*, de Bary, is seen in Fig. 50. The pyrenoids and nucleus have already divided, and the new cell-wall is beginning to form, whilst the daughter nuclei have migrated towards the constrictions in the chloroplasts.

In all the constricted species examined the process of cell-division is quite different from that in both *Closterium* and *Hyalotheca*. There is never any migration of the daughter nuclei, for these, in all the forms examined, naturally lie, as soon as formed, one at each end of the rapidly elongating isthmus between the two semi-cells of the dividing individual. Thus from the first they occupy their normal position in the isthmus, and as

the new semi-cells are formed there is no need for any change. In such forms it is rather the chromatophores which migrate.

Cell-division was very similar in all the Placodermæ investigated. The individual about to divide is usually very densely green, its chloroplasts are coarsely granular, and neither their definite structure nor the position of the pyrenoids can be distinguished. The reason for this is doubtless that the chloroplasts are very distended with stroma starch, which gives them the shapeless granular appearance, and by its great refractivity obscures the pyrenoids. The cytoplasm is sometimes crowded with numerous colourless oily-looking globules.

The division of the nucleus, accompanied by the elongation of the isthmus, and the subsequent formation of the transverse cell-wall are all completed before any visible changes take place in the chromatophores. The young semi-cells, still colourless, begin to round themselves off, and may even separate before anything further happens as far as the chromatophores are concerned. At this stage the protoplasm in the young semi-cells is becoming rather vacuolate, and its rapid streaming movements are clearly visible. Very often the daughter nuclei can be seen with high magnification as glistening bodies embedded in the protoplasm in the isthmus of each individual, and the colourless oily-looking globules may stream into the young semi-cells from the old one (Fig. 61). When the young semi-cells have attained a fair size, the chloroplast in each of the older half-cells begins to protrude slightly through the isthmus on each side (Fig. 62). This small part budded into the isthmus continues to increase in size as more and more of the chloroplast streams in from the old semi-cell into the rapidly growing young one. As the young semi-cell is gradually filled, the chloroplast contracts visibly from the wall of the older one (Figs. 62-5). Once the process has begun it continues very rapidly for a time; thus the period of time between the stages represented in Figs. 62 and 64 is only half an hour, and at the end of another half-hour the young semi-cells have begun to assume their characteristic form, and contain nearly as much of the chloroplast as the old ones (Fig. 65). The subsequent growth of the individuals was not so rapid, but in less than six hours after the beginning of the process the chloroplast had not only completed its budding, but in both old and young semi-cells had spread itself out so as to mantle completely the whole cell-wall, both individuals being now uniformly green (Fig. 67).

It now only remained for the chloroplast to divide at the isthmus. As observed in living specimens under the microscope this final process was very slow, and in many cases, in spite of the use of various arrangements to ensure a free supply of water between the slide and cover-glass, the individual often died before the chloroplasts had completed their division. In the specimen of *Euastrum Didelta* figured, the division was complete except for the final breaking of the thin strand connecting the two halves of

the chloroplast (Figs. 68-70), but the constriction of the chloroplast extended over two days. The complete division of the chloroplast was observed later in *Micrasterias denticulata*, in which the length of time between the stage represented in Fig. 69 and the final breaking of the drawn-out connecting thread was about sixteen hours. In their natural surroundings, however, it is most likely that the division of the chloroplast takes place far more rapidly than in specimens kept under observation under more or less unhealthy conditions. For in fixed and stained material one often encounters young individuals whose new semi-cells are not yet fully formed, yet whose chloroplasts have nevertheless completely divided at the isthmus.

It will be noticed that the actual division of the chloroplast differs somewhat from that previously described for *Closterium* and *Hyalotheca*. In both these genera there is a pinching-in of the chromatophore, and under the influence of the nucleus this furrow becomes deeper and deeper until the two halves are completely severed. In all the forms examined in this work such a pinching-in was not observed, the two halves of the chloroplast apparently pulling themselves apart, the connecting strand between them becoming thinner and thinner until it finally broke.

Owing to the dense nature of the cell-contents in most cases, and the large quantity of starch contained in the chromatophore, the behaviour of the pyrenoids could not usually be traced in the living condition, but in *Cosmarium subtumidum* it was possible under high magnification to distinguish the pyrenoids, and also to keep them under observation during the division processes.

In this species the single central pyrenoid, soon after the beginning of the budding of the chloroplast, begins to elongate slightly at its lower end (Fig. 71). The starch-grains rearrange themselves rapidly, and those at the lower end of the pyrenoid seem to form a small loop which doubtless contains a small globule budded off from the pyreno-crystal (Fig. 72). The latter cannot be seen, however, because of the great refractivity of the starch-grains. The small pyrenoid thus budded off from the original one apparently increases in size (Fig. 73), and the starch-grains arrange themselves so as to cut it off from the old one (Fig. 74). The process takes place very rapidly, ten to fifteen minutes sufficing for the complete division. During the formation of the new pyrenoid, the latter is gradually transported with the budding chloroplast into the young semi-cell (Figs. 71-4), but the two pyrenoids remain connected by means of starch-grains for a considerable time (Fig. 74).

In most of the species examined with more or less flattened cells, the chloroplast entered the young semi-cell through the isthmus as a somewhat bilobed protuberance (Fig. 62), although this form was sometimes quickly lost as the chloroplast streamed more and more into the new semi-cell. In the case of cells triangular or pentangular in the end view, and having nor-



mally a correspondingly three or five lobed chloroplast, the latter enters the new semi-cell from the old one as a three or five lobed mass (Figs. 49 and 55). Thus the definite form of the chloroplast is retained as far as possible during the process of budding, and, as seen in Fig. 55, the five-lobed form of the chloroplast is visible in the young semi-cells of *Staurostrum Brasiliense*, Nordst., var. *Lundellii*, West, at a very early stage, long before the characteristic shape of the cell-wall is apparent.

The nucleus of the cell is usually pushed to one side by the ingrowing chloroplast, especially where the latter is a single central one. In many cases it is very difficult, even in stained specimens, to distinguish the nucleus in newly divided individuals, in which the two halves of the chloroplast are not yet severed, but it can frequently be seen a little to one side (Figs. 49, 54, 56, 57, and 60). Occasionally it is carried away to some considerable distance into the new semi-cell by the intruding cell-contents, but whether such individuals survive in the end is doubtful.

The simple chloroplasts of the smaller species of *Euastrum* and *Cosmarium* offer no striking phenomena during cell-division. As in *C. sub-tumidum*, the single central pyrenoid constricts to form two during the process (Fig. 54). In the very numerous species of *Cosmarium* containing two chloroplasts in a semi-cell, each with one point of pyrenoid formation, the budding of the chloroplasts has already been figured by de Bary (1858) as typified by *C. Botrytis*. Each of the two chloroplasts bud into the new semi-cell through the isthmus, the pyrenoid of each meanwhile constricting to form two. The process is identical in *Euastrum verrucosum*, Ehrenb., and also in those species of *Xanthidium* having a similar chloroplast structure.

Where the axile chloroplast has more than one point of pyrenoid formation, as, for example, in *Staurostrum anatinum*, in which the cells, triangular in the end view, have typically one pyrenoid in each angle, all three pyrenoids constrict as the chloroplast projects as a trilobed mass into the young semi-cell (Fig. 58).

In the case of axile chloroplasts containing numerous scattered pyrenoids, e.g. *Tetmemorus*, *Micrasterias*, and *Staurostrum Brasiliense*, a number of pyrenoids are carried into the young semi-cell by the budding chloroplast (Figs. 56 and 60), and they do not conspicuously increase in number during the process.

With parietal chloroplasts, each one buds into the young semi-cell soon after its formation. Where each parietal plate contains a single pyrenoid, as in many species of *Xanthidium*, this constricts as the chromatophore projects through the isthmus, just as in the case of axile chloroplasts containing typically one pyrenoid; but if the pyrenoids are numerous and scattered, as in *X. armatum*, (Bréb.) Rabenh., a number of these pass into the new semi-cell along with the ingrowing chromatophore (Fig. 59).

In *Cosmarium Brebissonii*, Menegh., the complicated chloroplasts press into the young semi-cell as a comparatively shapeless mass, the pyrenoids thronging in at the same time (Fig. 57). In the figure the nucleus is seen pressed by the chloroplast to the right of the isthmus.

It was noticed that in the axile chloroplasts of many species there is a distinct tendency for the peripheral parts of the chromatophore to enter the young semi-cell very much more rapidly than the central part, as if there were an attempt on the part of the organism to cover the cell-wall with photosynthetic material as quickly as possible, no matter what happened in the interior of the cell. This is very pronounced in many species of *Staurastrum*, in which the angles of the young semi-cell may be quite filled by the chromatophore whilst yet the axial part of it has scarcely entered (Figs. 49 and 58). It is quite possible that very often the division of the chloroplast at the isthmus occurs before the axial part has entered the young semi-cell at all. Thus several distinct chloroplasts would be produced in the new half-cell instead of a single central one. In the next generation the entire cell would probably be provided with such chloroplasts in half the individuals.

This phenomenon has already been mentioned as being probably responsible for the frequent occurrence of wholly parietal chloroplasts in *St. grande* (Bulnh.), which normally possesses a single axile one, and also for the frequent shortening of the axis of the chloroplast in the median region in *Micrasterias truncata*, (Corda) Bréb., *M. oscitans*, var. *mucronata*, (Dixon) Wille, and *Cosmarium Ralfsii*, Bréb., and also for the production, in the extreme case, of two distinct chloroplasts in a semi-cell in the three latter species.

Further, in other species, one sometimes encounters odd specimens in which the chloroplasts, which normally should be axile, are more or less parietal. Such have been observed in *Tetmemorus Brebissonii*, (Menegh.) Ralfs, *T. granulatus*, (Bréb.) Ralfs, and others. The same explanation probably applies to these.

Again, it has been noted earlier that in the larger species of *Euastrum* a considerable proportion of individuals are found in which the axis of the chloroplast is either shortened, or else, together with the radiating plates, is altogether absent.<sup>1</sup> In extreme cases there may be several entirely parietal chloroplasts in a semi-cell. Unfortunately dividing specimens of the species concerned were not frequently met with in stained material, and so it was impossible to ascertain the real cause of the discrepancy, but it is not improbable that the abnormalities arose in young semi-cells during cell-division by the mantling of the cell-wall by the parietal plates long before the central axis and radiating plates had entered.

Finally, it is also suggested that in those species which normally possess

<sup>1</sup> Carter, N.: Studies on the Chloroplasts of Desmids. I. Ann. Bot., vol. xxxiii, 1919.

parietal chloroplasts, these were originally derived in a similar manner, and during the course of ages have become permanent.

Thus the fact that the chloroplast, during cell-division, streams so rapidly through a usually very narrow isthmus from the older semi-cell into the new one, offers many opportunities for variation in its form, and it is really surprising that in so many species the structure of the chloroplast is characteristic and constant. Considering that every chloroplast is derived from an original small bud of green material squeezed through a narrow passage one would expect a large proportion of abnormalities, and for this reason, at any rate, it would seem unwise to make the form of the chloroplast in this group the basis of a classification.

#### SUMMARY OF THE SPECIAL CHARACTERS OF *STAURASTRUM*.

Most species of *Staurastrum* have axile chloroplasts. The only species examined which always has parietal chloroplasts is *St. tumidum*.

Many of the smaller species have a simple axile chloroplast consisting of a central axis which contains a single pyrenoid and a bilobed mass projecting into each angle of the semi-cell.

Amongst the larger species the general form of the chromatophore is often quite similar to that of the smaller ones, but there are variations in the number of plates in each angle and also in the number and arrangement of the pyrenoids.

Most of the species examined had one point of pyrenoid formation in the centre of the semi-cell, but in a few species the pyrenoids occur either in the angles only, or else in addition to those in the centre.

*St. Brasiliense* and *St. grande* differ from most of the other species examined in their very numerous pyrenoids.

In *St. grande* also some individuals show a tendency to the parietal disposition of the chloroplast by the total disappearance of the axis in the centre of the semi-cell, leaving the peripheral lobes of the chloroplasts isolated.

#### SUMMARY OF THE CHARACTERS OF CELL-DIVISION.

The chloroplasts of *Netrium* and *Cylindrocystis* probably behave during cell-division in a manner essentially similar to that already described by Lutman for *Closterium*.

In all the Placoderm Desmids examined the process of cell-division is rather different from that of the Saccodermæ. The nucleus of the cell completes its division, and the two new colourless semi-cells can readily be distinguished before there are any visible changes in the chromatophores. The latter then rapidly stream through the isthmus from the old semi-cell into the new one, so that by the time it is fully formed it is usually uniformly green. The process is completed by the division of the chloroplasts at the isthmus of each individual.

In those species in which the points at which pyrenoids may occur are fixed the young semi-cell is provided with a corresponding number of pyrenoids by the budding of those already existing in the old semi-cell. Where the pyrenoids are indefinite in number and scattered, a number of these enter the new semi-cell together with the budding chromatophore.

A striking feature of the ingrowth of the chloroplast in many species is the rapidity with which the cell-wall of the young semi-cell is completely mantled by the chloroplast, often at the expense of the more central parts of the semi-cell. This phenomenon is responsible for the formation of parietal chloroplasts in isolated specimens of species which normally possess axile ones, and probably also for their original production in species in which they have been permanently acquired.

In conclusion I have to acknowledge the invaluable help and advice which I received throughout the whole of this investigation from the late Professor G. S. West, and also my indebtedness to him for providing much of the material. My thanks are also due to the Birmingham Natural History and Philosophical Society for a grant from their Endowment of Research Fund to help in the cost of producing the plates illustrating this work, and also to the Royal Society for a further grant for the same purpose.

THE BOTANICAL LABORATORY,  
UNIVERSITY OF BIRMINGHAM.

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## DESCRIPTION OF PLATES XIV-XVI.

Illustrating Miss Nellie Carter's paper on the Chloroplasts of Desmids. IV.

During the prolonged processes of preparation the specific characters of Desmids often become obliterated, but all the species were identified, either in the living or carefully fixed condition, by Professor G. S. West. The figures are only intended to indicate the structure of the chromatophores, and only the outlines of the cells are given, surface markings being omitted.

## PLATE XIV.

Figs. 1, 2. *Staurastrum forficulatum*, Lund.  $\times 510$ . Fig. 1, front view; Fig. 2, end view.  
 Figs. 3, 4. *St. orbiculare*, Ralfs.  $\times 510$ . Fig. 3, front view; Fig. 4, end view.  
 Figs. 5, 6. *St. Cerastes*, Lund.  $\times 510$ . Fig. 5, front view; Fig. 6, end view.  
 Figs. 7, 8. *St. gracile*, Ralfs.  $\times 510$ . Fig. 7, front view; Fig. 8, end view.  
 Figs. 9, 10. *St. Simonyi*, Heimerl.  $\times 510$ . Fig. 9, front view; Fig. 10, end view.  
 Figs. 11, 12. *St. punctulatum*, Bréb.  $\times 510$ . Fig. 11, front view; Fig. 12, end view.  
 Figs. 13, 14. *St. aversum*, Lund.  $\times 510$ . Fig. 13, front view; Fig. 14, end view.  
 Figs. 15, 16. *St. hirsutum*, (Ehrenb.) Ralfs.  $\times 510$ . Fig. 15, front view; Fig. 16, end view.  
 Figs. 17, 18. *St. jaculiferum*, West.  $\times 510$ . Fig. 17, front view; Fig. 18, end view.  
 Figs. 19, 20. *St. anatinum*, Cook and Wills.  $\times 510$ . Fig. 19, front view; Fig. 20, transverse section.

Figs. 21-3. *St. Sebaldi*, Reinsch, var. *altum*, (Boldt) West.  $\times 510$ , showing variation in number, size, and position of the pyrenoids. Fig. 21, front view; Fig. 22, oblique end view; Fig. 23, from the end.

Figs. 24, 25. *St. Sebaldi*, Reinsch, var. *ornatum*, Nordst.  $\times 510$ . Fig. 24, oblique front view; Fig. 25, end view.

Figs. 26, 27. *St. Ophiura*, Lund.  $\times 510$ . Fig. 26, front view; Fig. 27, transverse section.

Figs. 28, 29. *St. Arcticon*, (Ehrenb.) Lund.  $\times 510$ . Fig. 28, front view; Fig. 29, oblique end view. In both cases the letters *a a'*, *b b'*, *c*, &c., show the relation between the processes of the cell-wall.

Figs. 30-2. *St. paradoxum*, Meyen, var. *longipes*, Nordst.  $\times 510$ . Fig. 30, front view; Figs. 31 and 32, end view.

Fig. 33. *St. aculeatum*, (Ehrenb.) Menegh.  $\times 510$ . End view.

Figs. 34, 35. *St. Manfeldtii*, Delp.  $\times 510$ . Fig. 34, front view; Fig. 35, end view.

Figs. 36-8. *St. pyramidatum*, West.  $\times 510$ . Fig. 36, front view; Figs. 37-38, end view.

## PLATE XV.

Figs. 39-42. *St. grande*, Bulnh.  $\times 510$ . Fig. 39, oblique front view of specimen having several chloroplasts; Fig. 40, end view of a similar individual; Fig. 41, front view of individual having one chloroplast only; Fig. 42, end view of similar specimen.

Figs. 43, 44. *St. sexangulare*, (Bulnh.) Lund.  $\times 510$ . Fig. 43, front view; Fig. 44, end view.

Figs. 45, 46. *St. Brasiliense*, Nordst., var. *Lundellii*, West.  $\times 510$ . Fig. 45, front view; Fig. 46, transverse section.

Fig. 47, 48. *St. tumidum*, Bréb., front view.  $\times 510$ .

Fig. 49. *Staurastrum aversum*, Lund.  $\times 510$ . Showing the more rapid budding of the peripheral parts of the chloroplast into the young semi-cell as compared with that of the axis itself. The longitudinal stretching of the pyreno-crystal preparatory to its constriction to form two is also to be seen.

Fig. 50. *Cylindrocystis crassa*, de Bary.  $\times 915$ . The pyrenoid of each chloroplast and the nucleus of the cell have already divided, whilst in the peripheral part of each chloroplast a transverse cleft has appeared into which a daughter nucleus is pressing its way, and the transverse wall is beginning to form. A number of proteid granules or small pyrenoids can also be seen in the peripheral parts of each chloroplast.

Figs. 51, 52. *Netrium oblongum*, (de Bary) Lütken., var. *cylindricum*, W. and G. S.

West.  $\times 510$ . Fig. 51 shows the division of the pyrenoid and constriction of the chloroplast preparatory to cell-division; Fig. 52 shows the completed division.

Fig. 53. *Netrium Digitus*, (Ehrenb.) Itzigs and Rothe.  $\times 510$ . A stage in cell-division intermediate between Figs. 51 and 52, showing the travelling of the two daughter nuclei towards the clefts between the newly divided chloroplasts.

Fig. 54. *Euastrum bidentatum*, Näg.  $\times 510$ . Showing division of the pyrenoid during cell-division.

Figs. 55, 56. *Stauroastrum Brasiliense*, Nordst., var. *Lundellii*, W. and G. S. West.  $\times 510$ . Fig. 55, very young semi-cell seen from the end, showing that the ingrowing chloroplast even at this early stage preserves its five-lobed structure, although the pentangular shape of the cell-wall itself is scarcely to be discerned; Fig. 56, front view of a much later stage, showing the entrance of the pyrenoids from the old semi-cell, and the nucleus displaced to one side.

Fig. 57. *Cosmarium Brebissonii*, Menegh.  $\times 510$ . Side view of dividing specimen, showing the ingrowth of the chloroplast and pyrenoids, and the nucleus pushed to one side.

Fig. 58. *Stauroastrum anatinum*, Cook and Wills.  $\times 510$ . Front view of dividing individual, showing the division of the pyrenoids and the rapid growth of the more peripheral parts of the chloroplasts as compared with that of the more central part.

Fig. 59. *Xanthidium armatum*, (Bréb.) Rabenh.  $\times 510$ . Front view of a very early division stage, showing the beginning of the budding into the young semi-cell of the chloroplast together with its pyrenoids.

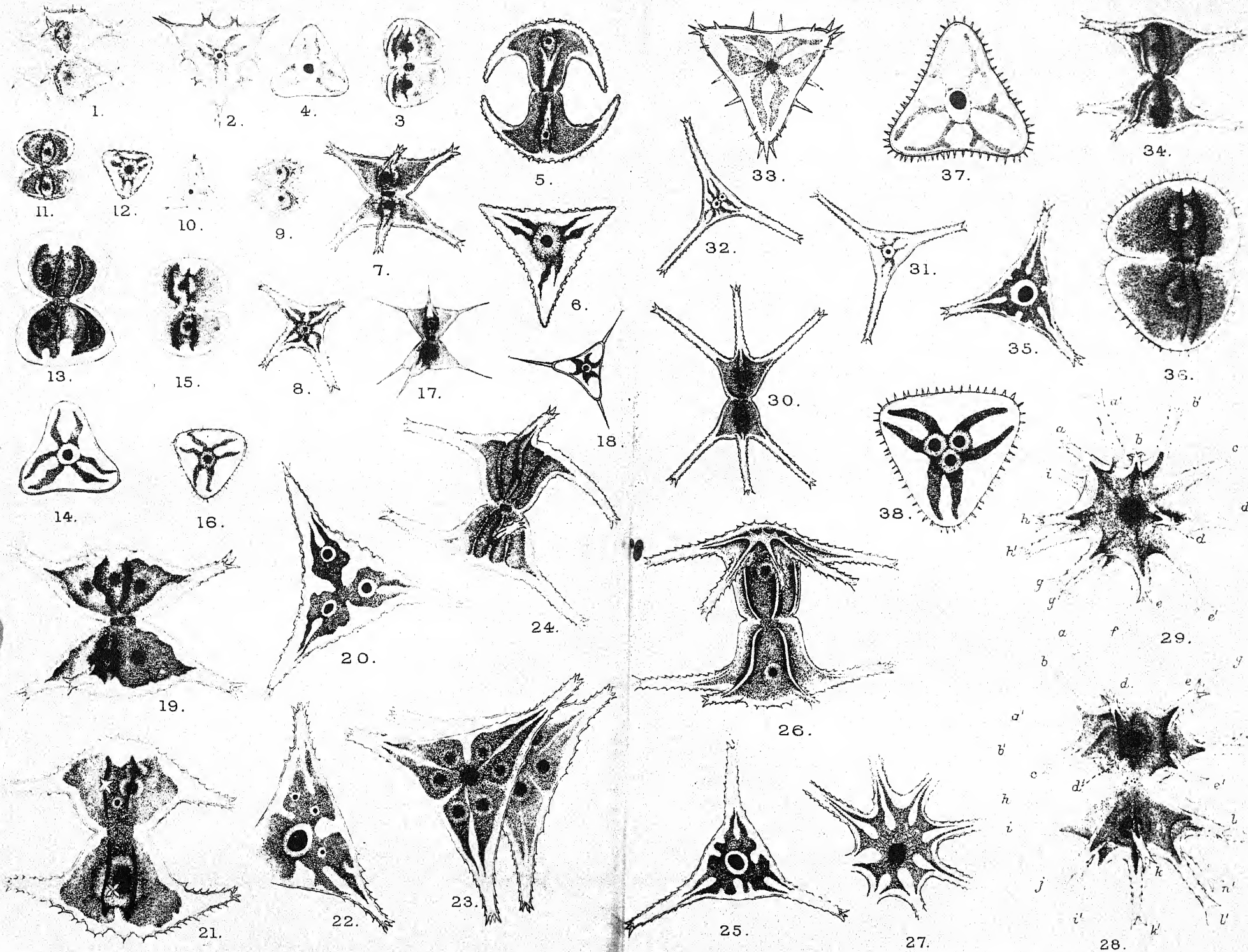
Fig. 60. *Tetmemorus granulatus*, (Bréb.) Ralfs.  $\times 510$ . Side view of a recently divided specimen in which the chloroplast has entered the young semi-cell, but division at the sinus has not yet taken place.

#### PLATE XVI.

Figs. 61-70. *Eu. Didelta*, (Turp.) Ralfs.  $\times 350$ . Process of cell-division followed in a single individual. Fig. 61, July 25th, 11.15 a.m.; Fig. 62, 11.30 a.m.; Fig. 63, 11.40 a.m.; Fig. 64, 12 noon; Fig. 65, 12.30 p.m.; Fig. 66, 2 p.m.; Fig. 67, 5 p.m.; Fig. 68, July 26th, 10.30 a.m.; Fig. 69, 11.30 a.m.; Fig. 70, July 27th, 10.30 a.m.

Figs. 71-4. *Cosmarium subumidum*, Nordst.  $\times 1,750$ . Behaviour of the pyrenoid during cell-division. Fig. 71, 3.20 p.m.; Fig. 72, 3.25 p.m.; Fig. 73, 3.30 p.m.; Fig. 74, 3.37 p.m.



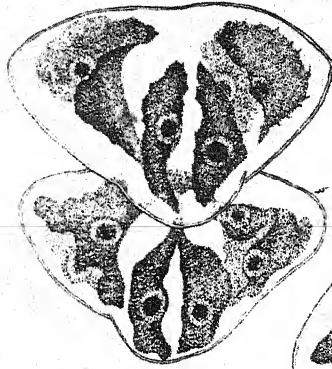


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CARTER-CHLOROPLASTS OF DESMIDS.

Huth, London.

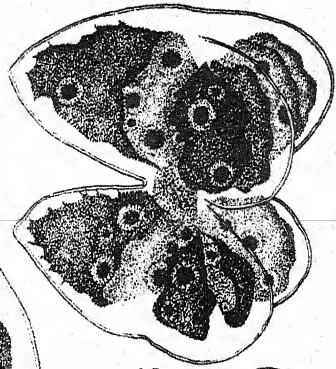




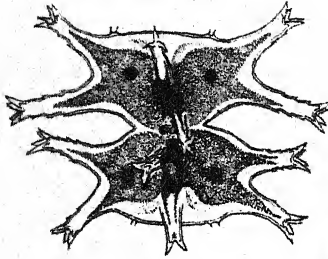
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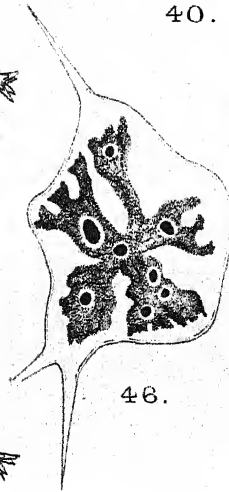
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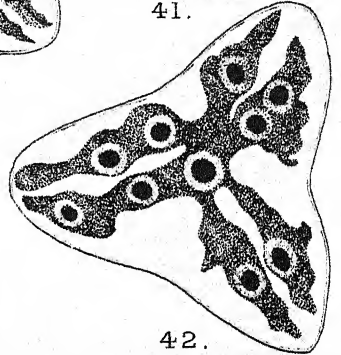
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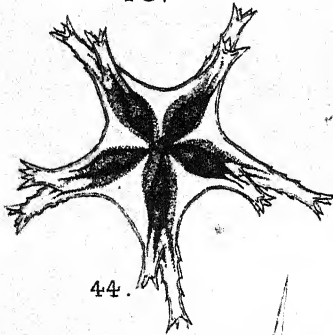
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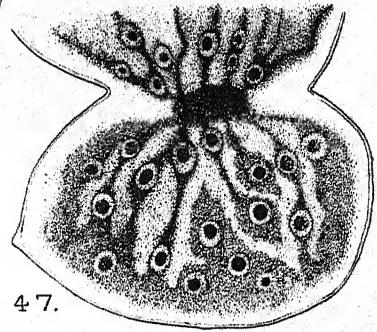
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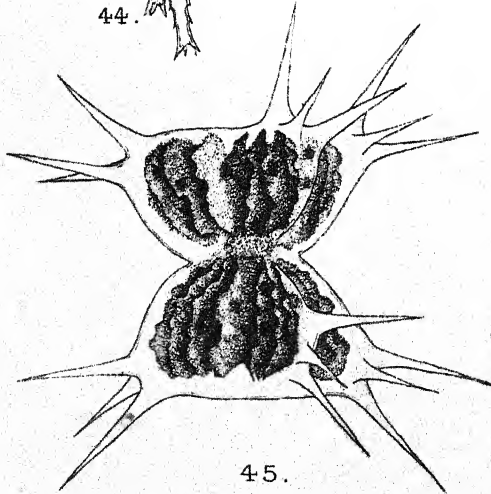
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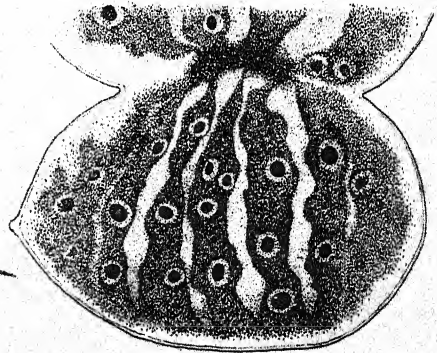
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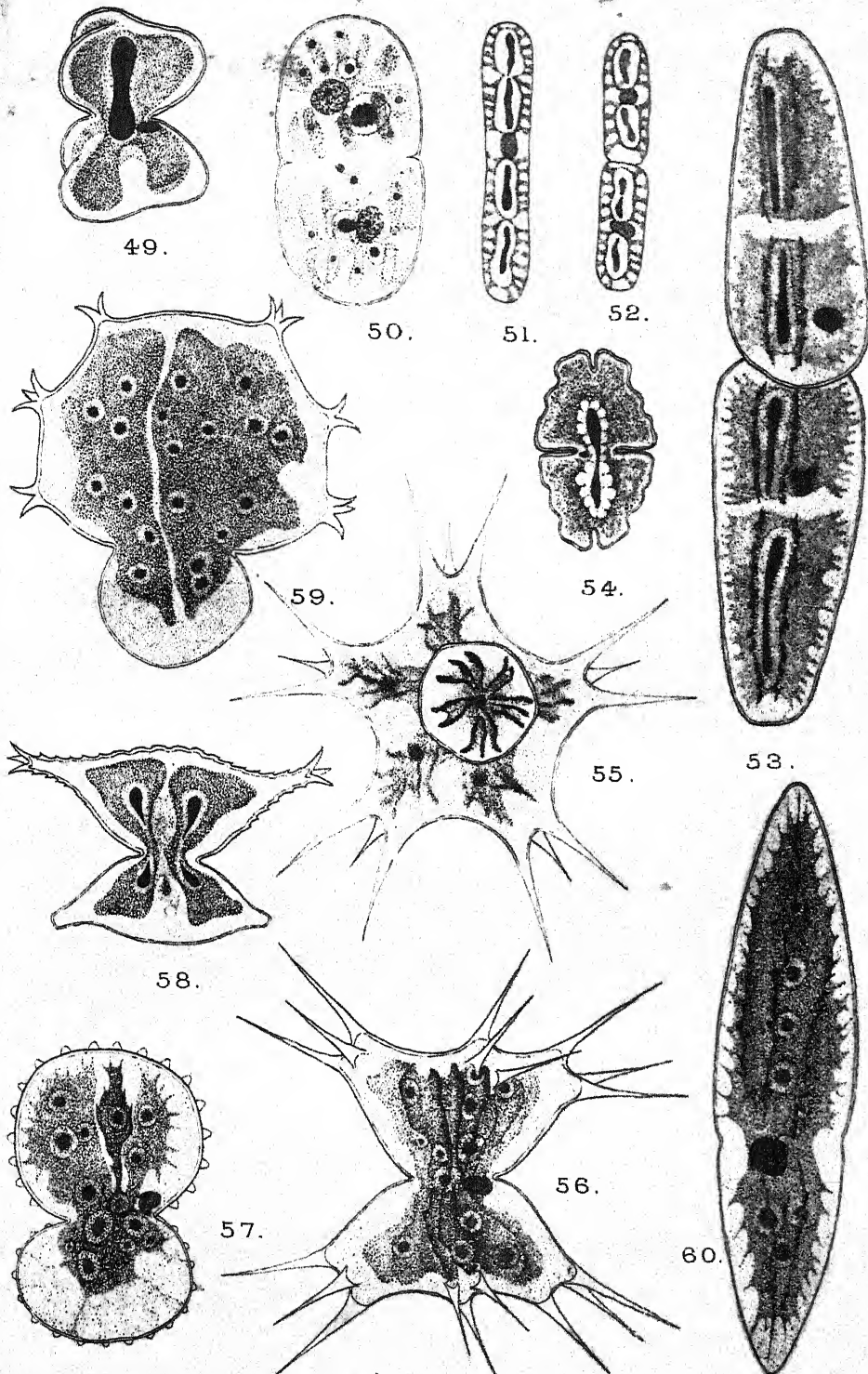


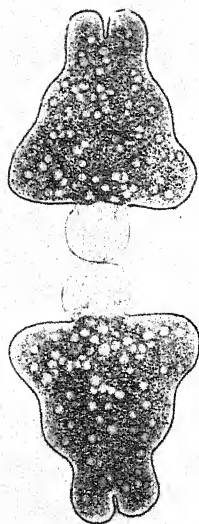
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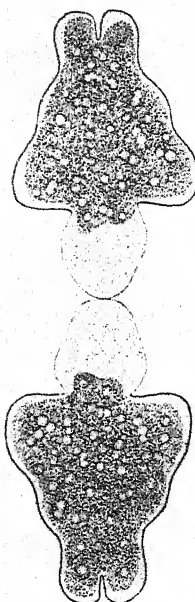
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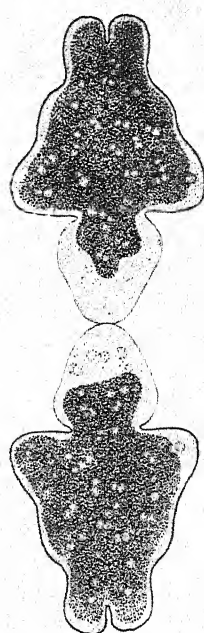




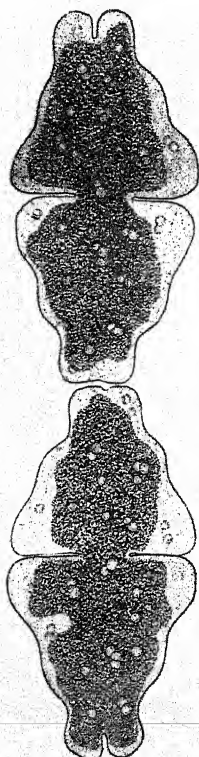
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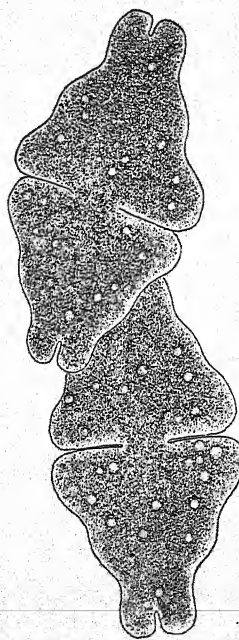
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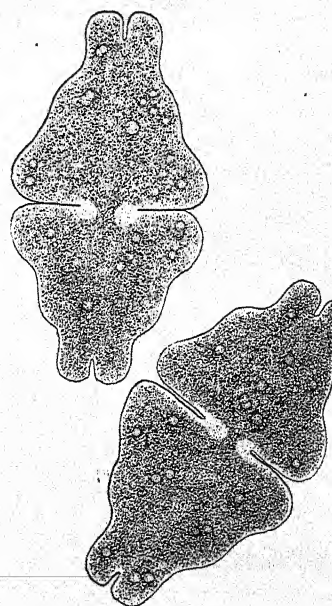
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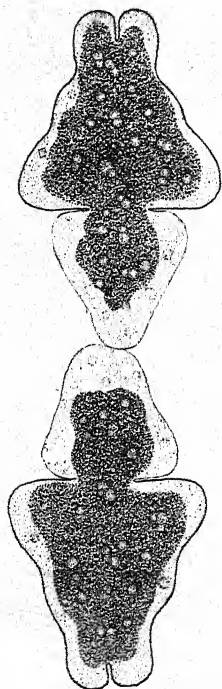


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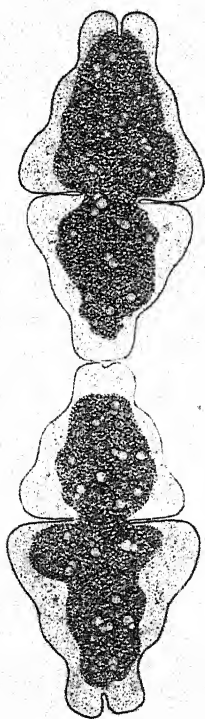


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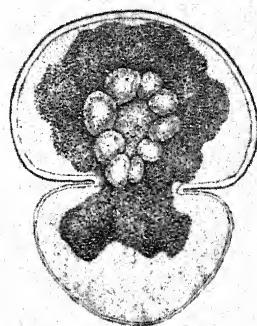




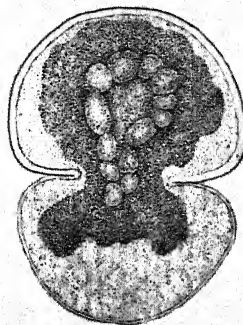
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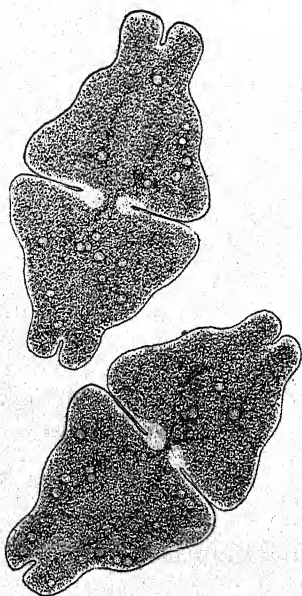
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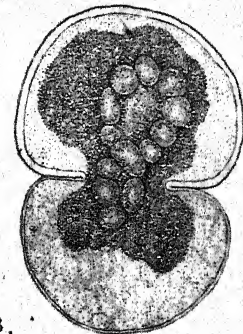
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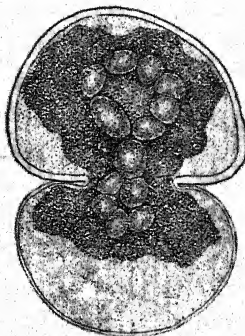
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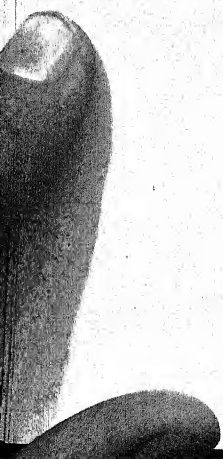
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# Observations on the Anatomy of Teratological Seedlings.

## III. On the Anatomy of some Atypical Seedlings of *Impatiens Roylei*, Walp.

BY

H. S. HOLDEN, M.Sc., F.L.S.,

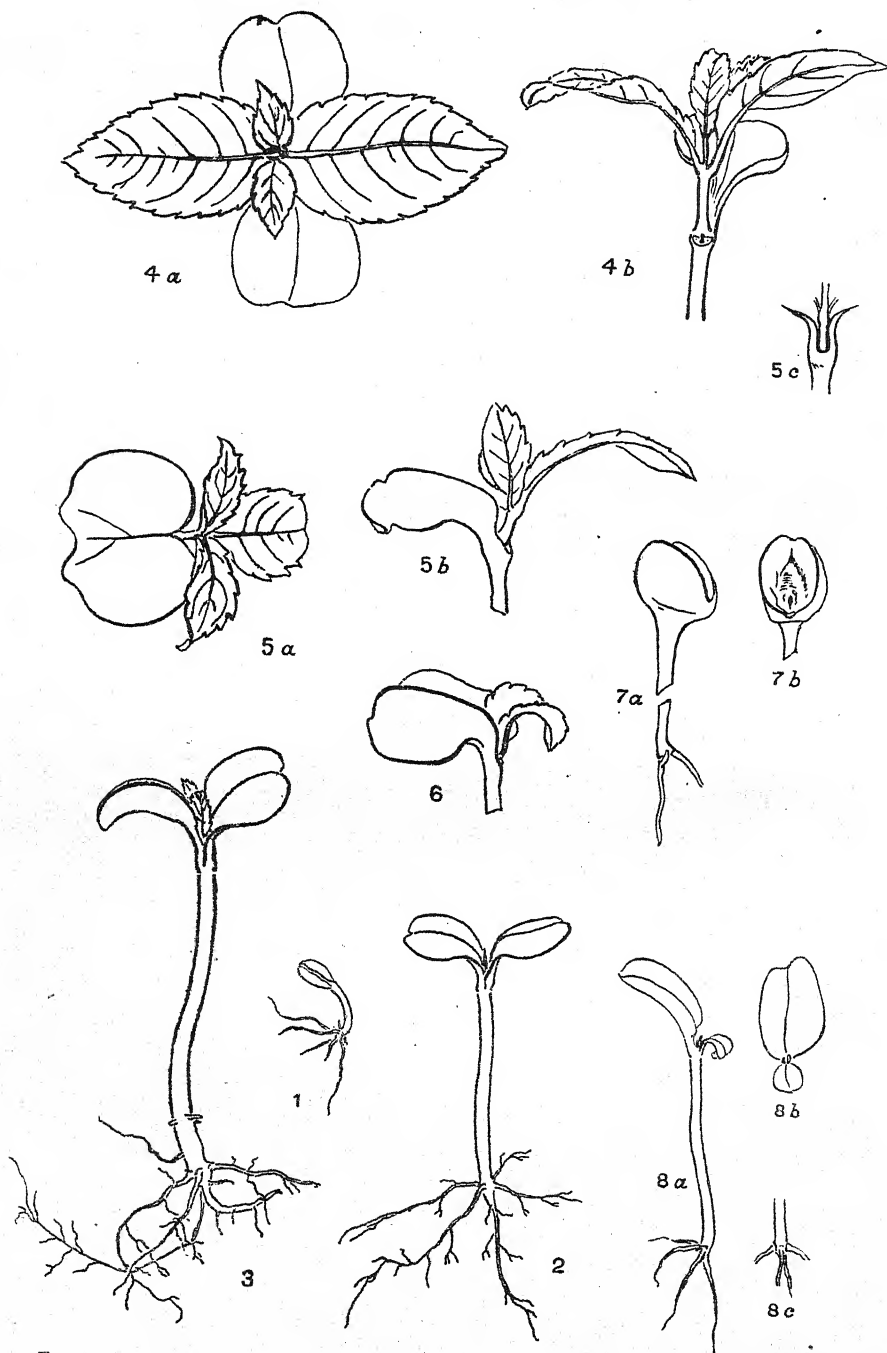
*University College, Nottingham.*

With one hundred and thirteen Figures in the Text.

*IMPATIENS ROYLEI* is an annual of Indian origin which has become naturalized in England, and which, when once introduced, rapidly colonizes patches of bare ground unless thoroughly eradicated. A plot of ground in the garden of the University College, Nottingham, is completely covered with the plant in question, and it is from this source that much of the material for the present investigation has been derived. A further supply of seedlings has been obtained from the gardens of friends in Plymouth, the total number examined being sixty-one. It is proposed first of all to give some account of the vascular anatomy of the normal seedling and the young epicotyl, and then to show how these have been modified in the atypical specimens studied.

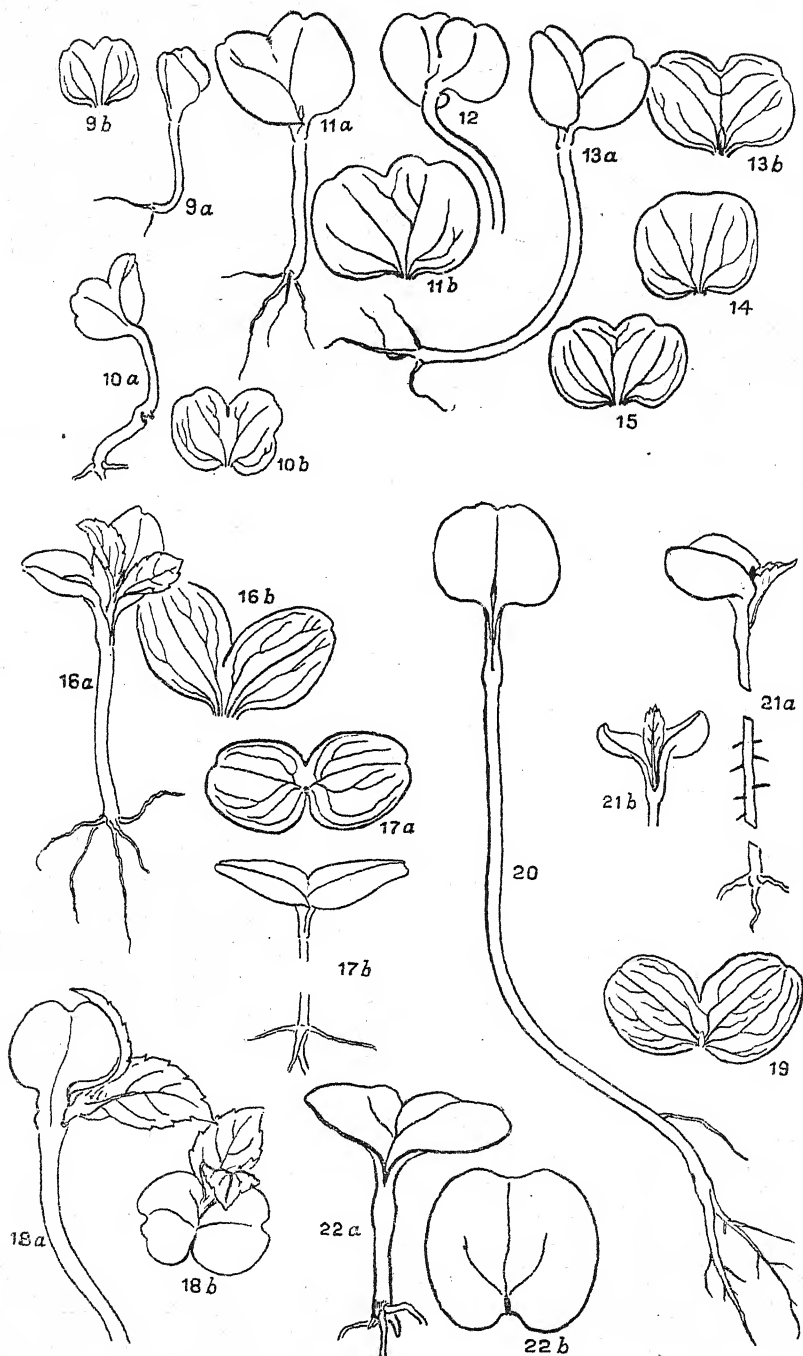
1. *The hypocotyl of the seedling*, at the time that the first epicotyledonary leaves become obvious, is about five centimetres in length and bears two somewhat fleshy, heart-shaped cotyledons with stout petioles which occasionally unite basally to form a very short cotyledonary tube. At the junction of the hypocotyl and root is a whorl of four stout rootlets below which the main root thins considerably (Figs. 1, 2, and 3). This whorl of roots, which is initiated at a relatively early stage in the development of the embryo, is very characteristic and forms a useful basis of comparison in studying the abnormal seedlings. A similar root whorl has also been described in *Impatiens Balsamina* by Chauveaud (1, p. 334).

In most seedlings the vascular supply of each cotyledon consists, at the proximal end of the blade, of a median endarch xylem group with a mass of phloem on either flank, and a pair of lateral bundles; this system giving rise to a tetrarch hypocotyledonary grouping, and in the root to a solid tetrarch xylem star with the usual radial arrangement of the phloem. In the very young seedling (Fig. 1) exarchy is evident a little way



FIGS. 1-8. Figs. 1, 2, 3, successive stages in the growth of the normal seedling, showing the whorl of four lateral roots at the junction of hypocotyl and tap-root. Fig. 4a, older seedling viewed in plan, showing the relationship of the cotyledons and the epicotyledonary leaves at the first two nodes. Fig. 4b, the same in lateral view, one cotyledon removed. Figs. 5a, b, c, different aspects of a typical syncotyl for comparison with Figs. 4a, b. Fig. 6, side view of a Group II seedling with peltate cotyledon. Figs. 7a, b, two views of a Group II seedling. Figs. 8a, b, c, seedling with unequal cotyledons and a whorl of three asymmetrically placed rootlets.





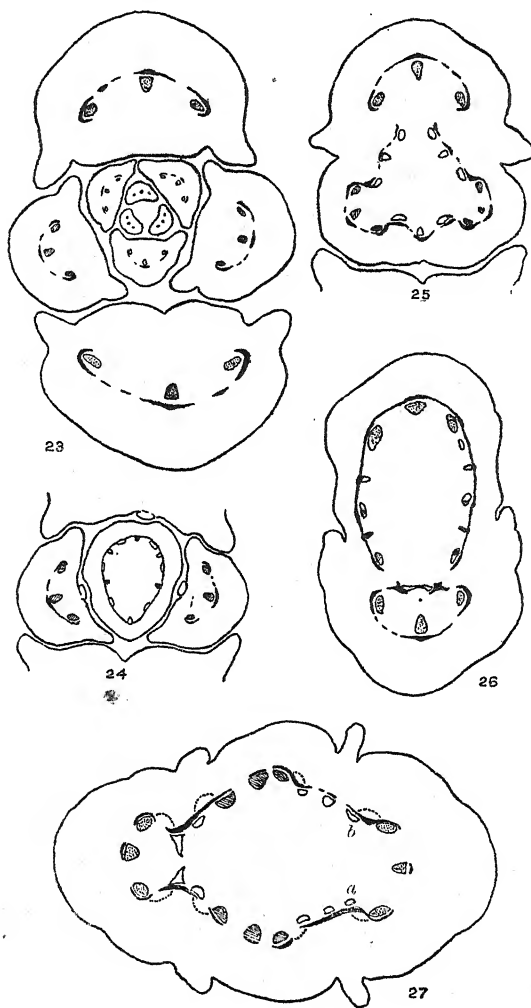
FIGS. 9-22. Figs. 9-19, typical examples of syncotyls showing modification of general habit and venation. Figs. 20, 21 *a*, *b*, typical Group II seedlings. Note the two rows of hypocotyledonary roots arising from the protoxylem poles in Fig. 21 *a*. Figs. 22 *a*, *b*, Group II seedling with a whorl of four roots and a tetrarch symmetry.

below the apex of the hypocotyl, so that four exarch xylem groups, with alternating phloem, traverse the hypocotyl until the base is approached. This primary structure becomes very considerably modified in older seedlings, owing to secondary changes. Prior to the giving off of the whorl of lateral roots, to which reference has been made earlier, the typical vascular structure of the hypocotyl is obscured, even in the youngest seedlings examined, by the development of a solid plug of xylem consisting of short tracheidal elements, and this persists until it is gradually replaced by the tetrarch xylem star characterizing the main root (cf. Fig. 99).

2. *The vascular system of the young epicotyl.* In the normal seedling the leaves borne at the first two epicotyledonary nodes are decussate and opposite, the lower pair being in the intercotyledonary plane: those produced at subsequent nodes are usually in whorls of three. Although the anatomy is subject to minor variations the essential features are stereotyped, and it is proposed to describe the course of the vascular strands from above downwards in a typical specimen at a stage in which two *whorls* of leaves are developed. For the sake of brevity the leaves will be referred to as the upper and lower whorls and the upper and lower pairs respectively. The petiolar vascular supply consists of three collateral bundles, one median and two lateral, the latter often being somewhat flattened transversely. There is thus at the apex of the young stem a ring of nine bundles (Fig. 23). This number becomes doubled by the entry of the three bundle-systems of the lower whorl, the eighteen bundles thus produced being reduced, first to twelve by the fusion of the laterals (Fig. 24), and then to nine again by the subsequent union of the median bundles of the upper whorl with one of the adjacent compound laterals (Fig. 28). This fusion of the median bundles is generally regular in either a clockwise or a counter-clockwise direction, but in some specimens it is irregular, two fusing in a clockwise direction whilst the third fuses in the opposite direction, or vice versa. The insertion of this trimerous system on to that of the members of the upper and lower pairs gives rise to an interesting disturbance of symmetry. The bundle-systems of the upper pair do not enter the stem exactly in the cotyledonary plane, but slightly obliquely, forming with those of the whorl above an irregular ring of fifteen bundles (Figs. 24, 25). This number is ultimately reduced by the fusion of the adjacent laterals, but often before this occurs an additional complication arises owing to the insertion of the components from both nodes on to the vascular bundles of the lower pair.<sup>1</sup> The latter enter the stem in the intercotyledonary plane, but one group shows a retarded rate of entry compared with its fellow, a feature which is to be correlated with the difference in the methods of insertion of the vascular strands from

<sup>1</sup> The level at which the fusion of the adjacent laterals occurs is somewhat variable. It may occur soon after the entry of the bundles of the upper and lower pairs into the stem (Fig. 26), or it may be delayed until a relatively late stage (Fig. 27).

above. It will be perceived that *two* of the *lateral* bundles of the lower whorl (*a* and *b*, Figs. 27, 28) have of necessity no part in the bundle fusions occurring at the second epicotyledonary node, and it is these which unite



FIGS. 23-27. The distribution of the vascular strands in the young epicotyl. Fig. 27 is from an older seedling than the rest. In Fig. 26 the laterals of the lower whorl have, with the exception of the upper one on the right, fused with the adjacent laterals of the upper and lower pairs respectively. Bundles from the lower whorl are indicated in outline, from the upper pair cross-hatched, from the lower pair dotted. The dotted lines in Fig. 27 indicate the bundles which first unite. These and all subsequent figures, except Fig. 28, are camera lucida drawings reduced to scale.

with the lateral bundles of that one of the basal pair which first enters the stem (Figs. 25, 27, 28). The laterals of the other member of the basal pair each unite with a half-bundle derived from the midrib of that leaf in the lower whorl standing vertically above it, this bifurcating to produce the

necessary gap (Figs. 26, 27, 28). A transverse section of the epicotyl at this point will therefore, if no prior lateral fusions have occurred, reveal a system of twenty-two bundles comprised as follows:

- (a) Two sets of three from the lower pair of leaves;
- (b) Two sets of three from the upper pair of leaves;
- (c) Two sets of three from two of the leaves of the lower whorl;
- (d) Four bundles from the remaining leaf of the lower whorl;

namely, the laterals and bifurcated midrib (Fig. 27), this number being reduced to fourteen by the lateral fusions to which reference has already

been made. A further reduction to twelve bundles is next produced by the fusion of each of the two undivided median bundles from the lower whorl with one of the laterals of the adjacent leaf of the basal pair (Fig. 28). The twelve bundles are reduced to six by the unequal fusion of those of the upper pair of leaves with those of the lower pair, the median bundle and one of its laterals fusing with one set of lower pair laterals, whilst the remaining laterals of the upper pair fuse with the other set of lower pair laterals. A certain compensatory principle is observable in this unequal fusion, the two *pairs* of bundles fusing with the laterals which received the products of the bifurcating strand, whilst the remaining bundle fuses with the laterals which received two bundles each at the previous node. The six bundles so formed enter the apex of the hypocotyl in the intercotyledonary plane and eventually fuse to form two xylem

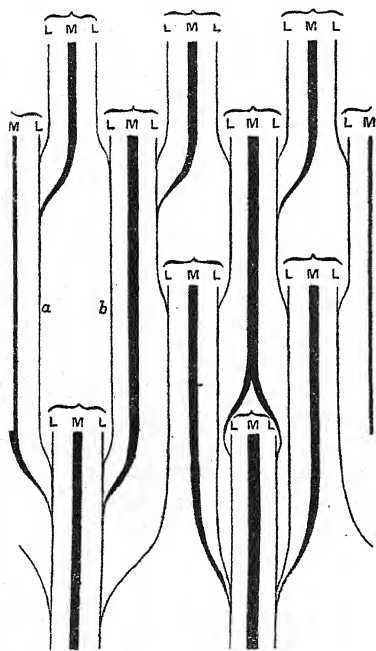


FIG. 28. Diagram indicating the course of the bundles in the young epicotyl.

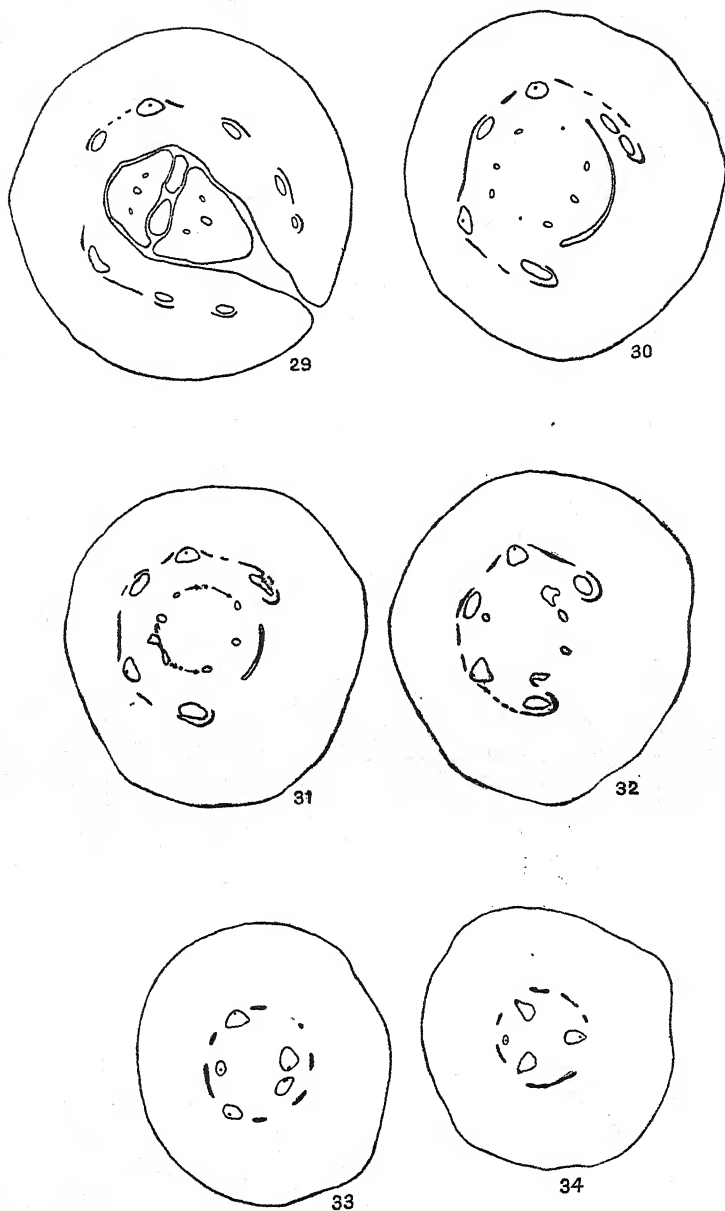
masses which, in fairly old seedlings, are continuous with a patch of secondary xylem immediately below them. The relationship of the epicotyledonary strands to the vascular strands of the cotyledons is also a feature of some interest, especially in mature seedlings.<sup>1</sup> As the latter approach the former they appear in transverse section as bow-shaped systems, the ends of which extend beyond the laterals from the epicotyl. At the same time connexion is established between the two by the phloem

<sup>1</sup> By mature seedlings is meant those in which vigorous epicotyledonary growth has begun and in which the cotyledons have attained their full development.

of the cotyledonary laterals travelling round the outer ends of the bundles and uniting with that of the epicotyledonary laterals. At a slightly later stage the phloem, in some seedlings, forms locally a ring broken only opposite the median cotyledonary bundles. Simultaneously the epicotyledonary and cotyledonary xylems are brought into close contact by a collar of transfusion tracheides extending across the inner faces of each of the latter. The identity of the epicotyledonary xylem is ultimately lost in the previously mentioned patch of secondary xylem.

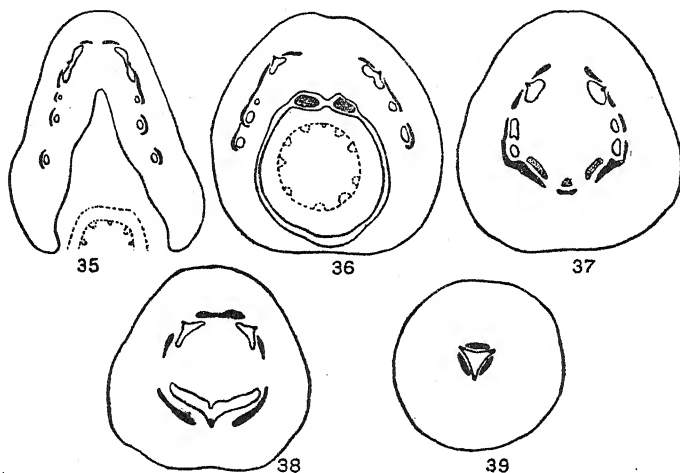
3. *The atypical seedlings*, with one exception, fall into two well-defined groups. The first of these comprises forty-three seedlings and forms a very complete series illustrating the development of a closely syncotylous condition from the normal.

Accompanying this development there has been a compression modification of the epicotyl, though the two processes have not proceeded with absolute uniformity. In all cases but one the syncotily is essentially unilateral, although a short cotyledonary tube is very commonly formed. The exceptional specimen shows a fusion of the proximal third of the cotyledonary laminae and the distal parts of the petioles by their upper surfaces (Figs. 18 *a*, 18 *b*). The resultant modifications in this instance have been relatively slight, consisting of a lateral displacement of the epicotyl and a distortion of one of the lateral cotyledonary bundles which is involved in the fusion and which appears to have lost its phloem locally. Of the remainder the simplest case is one in which the cotyledonary fusion is purely laminar (Fig. 12) although the petioles are closely apposed throughout. Both in this and in such cases as those in which the petioles only are completely fused and in which the major portions of the laminae are free (Figs. 16 *a*, 16 *b*) the normal tetrarch symmetry of the vascular system remains undisturbed. In one seedling of this type, however, a curious condition is brought about by the persistence of the epicotyledonary xylem, which, lying in the intercotyledonary plane, constitutes a barrier between the apposed lateral bundles, preventing their fusion at the apex of the hypocotyl. The separation is maintained for quite a third of the way down the hypocotyl, and during that time the lateral bundles are endarch and offer a striking contrast to the exarch median bundles. Ultimately they unite, coming together at a very acute angle and thus differing from the sharp, almost horizontal fusion which is normal for the species. With regard to the modifications in the epicotyl, beyond a slight and variable reduction in the size of the leaf on the fusion side at the first node, these are usually negligible. One very definite tendency must, however, be noted, and that is that the vascular systems from the second epicotyledonary node incline to a precocious fusion with that of the reduced leaf, this often occurring prior to the entry into the stem of the vascular system of the unaffected leaf. As the laminae become more closely implicated in the



FIGS. 29-34. Syncotyl showing the suppression of one lateral bundle on the symphysis side and the compression of the leaves at the second epicotyledonary node. Note the modification of the bundle distribution in the epicotyl.

fusion the effect of the ensuing compression on the laterals on that side becomes plainly evident (cf. Figs. 10 *b*, 11 *b*, 13 *b*, 14, 15, 16 *b*, 17 *a*, and 19). In one or two instances both persist in a much reduced condition, but do not form a root pole, and end blindly in the hypocotyl. More usually only one persists, and, though in one instance (Figs. 29–34) it remains prominent through the greater part of the hypocotyl and becomes mesarch, it ultimately dies out. Generally the persistent lateral is represented in the hypocotyl by a few metaxylem elements which have no effect on the trimerous symmetry which has resulted from syncotily. In the lamina it is usually well-developed and often receives bundles from both cotyledons (Figs. 13 *b*, 15, 19). The result of the further stages in syncotily is to cause its complete suppression (Figs. 35–39), this being compensated to some extent by



FIGS. 35–39. Typical advanced syncotily showing suppression of both laterals on the symphysis side and delay in the fusion of the lateral and marginal bundles. Note the two cotyledonary buds in Fig. 36.

a slight elaboration of the lateral bundle-system of both cotyledons on the side remote from the symphysis (Figs. 35, 36). The other points common to the whole series are that where the seedling is sufficiently developed two cotyledonary buds are always present, and also that a short cotyledonary tube is practically a constant feature (Fig. 36).

Reference has already been made to the precocious epicotyledonary vascular fusions incidental to the less extreme cases of syncotily. This stage is followed by one showing a displacement of the vascular supply of the leaf of the first epicotyledonary node situated on the symphysis side, which, instead of retaining its normal position in the intercotyledonary plane, divides into two parts, one consisting of the midrib and one lateral bundle, and the other of a lateral bundle only. These fuse with the right and left laterals of the other leaf from the same node, so that the vascular supply of



the epicotyl below that point resembles an open gutter with its concavity towards the point of cotyledonary symphysis (Figs. 40-43). This condition characterizes the majority of those syncotyls in which there is partial suppression of the involved lateral cotyledonary bundles, and where the suppression is complete this is accompanied in many cases by a corresponding suppression of the first epicotyledonary leaf on the same side. As has been stated, however, the cotyledonary and epicotyledonary modifications do not synchronize exactly, so that complete suppression of the epicotyl leaf on the symphysis side may occur with a still persistent cotyledonary lateral, whilst a retention of the leaf in a reduced state may characterize a seedling in which the laterals have disappeared. Only one instance was observed in which compression affected the paired leaves of the second epicotyledonary node, and in this seedling, which was one with a single persistent lateral, the leaves of the first epicotyledonary node were practically normal in size, whilst the affected leaves were markedly reduced (Fig. 29). The behaviour of the vascular strands of the symphysis leaf was curiously aberrant, one of its lateral strands bifurcating and half fusing with the median strand to form a compound bundle situated close to one of the cotyledonary strands. This persisted for some time independently and ultimately died out, whilst the other half-lateral and the lateral on the opposite side formed the extreme members of the usual gutter-shaped system (Figs. 30-32).

What may possibly represent the ultimate effect of compression on the epicotyledonary leaves is seen in some of the seedlings in which the cotyledonary laterals on the symphysis side are completely suppressed. In these seedlings the number of leaves at the third cotyledonary node is reduced to two lying in what is normally the intercotyledonary plane (Fig. 49; cf. also Fig. 54). The vascular system of the one standing vertically above the persistent leaf from the first node behaves similarly to that occupying the same position in typical dicotyls, its median bundle bifurcating and the constituent halves uniting with the laterals of the basal leaf, whilst its laterals unite with the adjacent bundles from the leaves of the second node. The bundles of the other leaf from the third node unite with the adjacent laterals of the leaves of the second node, its midrib and one lateral fusing with one second node lateral, and its remaining lateral with the other. Their behaviour thus resembles that of the bundles of the leaf of the first node on the symphysis side in those seedlings in which it persists in a reduced condition (Figs. 51, 52; cf. also Figs. 54-59).

The effects of progressively closer syncotyl may therefore be summarized as follows:

A. Cotyledonary vascular system.

(i) Modification of the lateral bundles on the symphysis side leading to their reduction, either simultaneously or otherwise, and their ultimate suppression.

(ii) Reduction in the hypocotyl and root of the tetrarch to triarch symmetry.

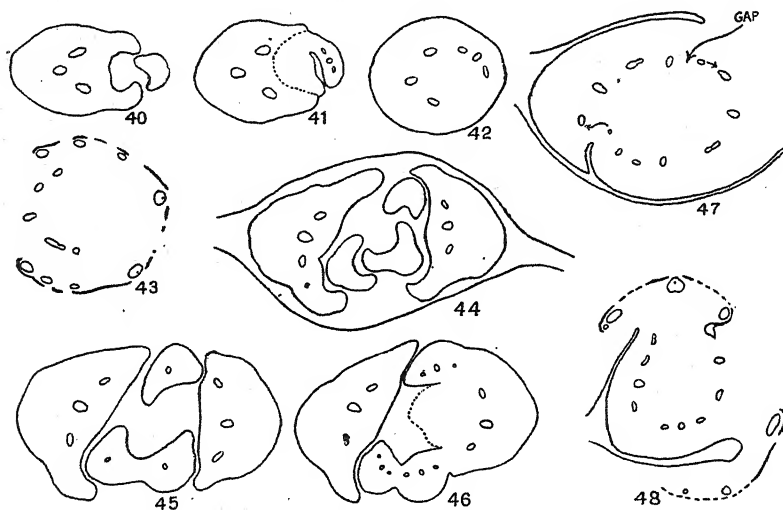
B. Young epicotyl.

(i) Precocious fusion of the bundle-systems of the leaves of the second node on to that of the leaf of the basal node on the symphysis side.

(ii) Reduction of the leaf of the basal node on the symphysis side and the division and lateral displacement of its vascular system.

(iii) Complete suppression of this leaf, and possibly

(iv) Reduction in the number of leaves at the third node from three to two with modification of the vascular relationships of one of the persistent members.

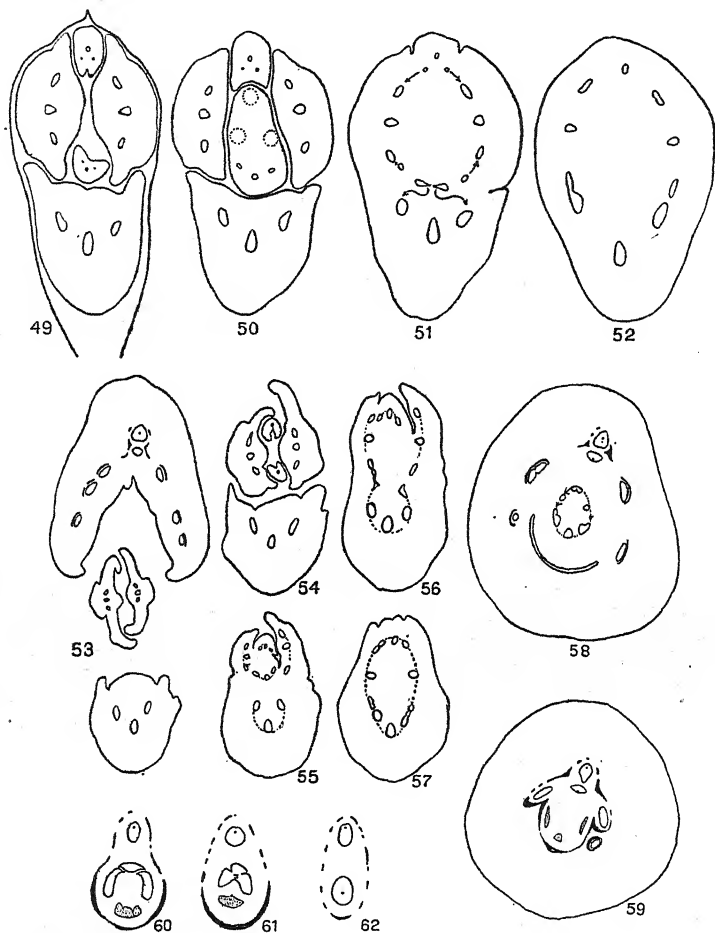


FIGS. 40-48. Figs. 40-43, young epicotyl in syncotyl showing reduction of one leaf of the basal pair and the modification of its bundle distribution. Figs. 44-48, young epicotyl of seedling with unequal cotyledons (Fig. 8) showing bundle distribution. Note the trimerous whorl at the second node and the precocious fusion of two of its members.

Comparing these results as regards the cotyledon with those obtained by Compton (3) in his study of syncotyls, it will be seen that up to a point the correspondence between the two is very close, but that the ultimate stages recorded by him for *Helianthus annuus syncotyleus*, namely, partial union of the median bundles and, more doubtfully, partial suppression of one of the median bundles, are absent in the material of *Impatiens Roylei*. Compton apparently made no attempt to study the effect of advanced syncotyl on the young epicotyl.

The second group of seedlings, seventeen in number, also form a very completely graded series. All the members of this group show what is apparently a single cotyledon with no macroscopic evidence of syncotylous origin (Fig. 20). The first epicotyledonary node bears a single leaf

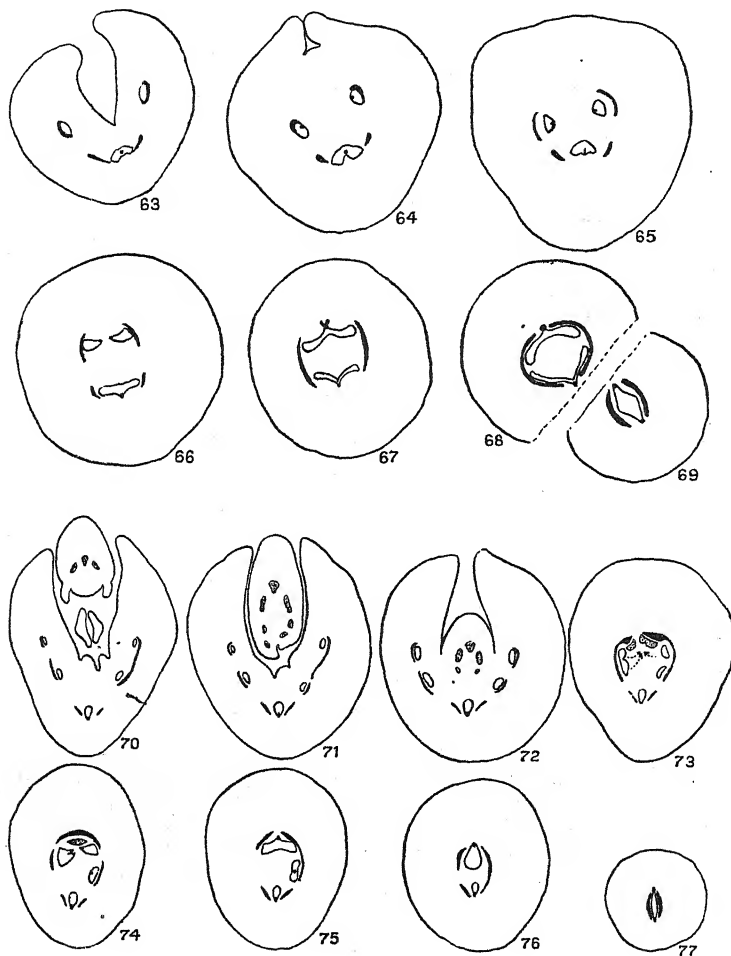
occupying what in a normal seedling would be the place of a second cotyledon (Fig. 6). In the simplest case the anatomy of the cotyledon is identical with that of one cotyledon of a typical dicotyl, its vascular supply consisting of a median 'double' bundle with a lateral on either flank. The behaviour



FIGS. 49-62. Figs. 49-52, epicotyl of syncotyl showing total suppression of first epicotyledonary leaf on the symphysis side and two leaves only at the third node. Note the trimerous whorl at the fourth node. Figs. 53-57, a similar type of epicotyl in a Group II seedling. Figs. 58-62, cotyledon bundles from the same seedling, showing anomalous bundle situated in the same vertical plane as the double bundle. Note the development of diarchy by the concentration of the lateral and marginal bundles on the anomalously situated bundle.

of the median bundle is perfectly regular and it forms a root pole in the usual way. The two lateral bundles on entering the hypocotyl rapidly approach each other and unite to form a second root pole, so that a diarch condition obtains throughout the hypocotyl and root (Figs. 63-69). As a consequence the root whorl at the base of the hypocotyl is reduced to two

members, and it is of interest to note that the hypocotyledonary adventitious roots which characterize this plant, and which arise from the protoxylems, also develop in two vertical series instead of the usual four (Fig. 21 a). Starting from this basal type there has been a progressive elaboration of the



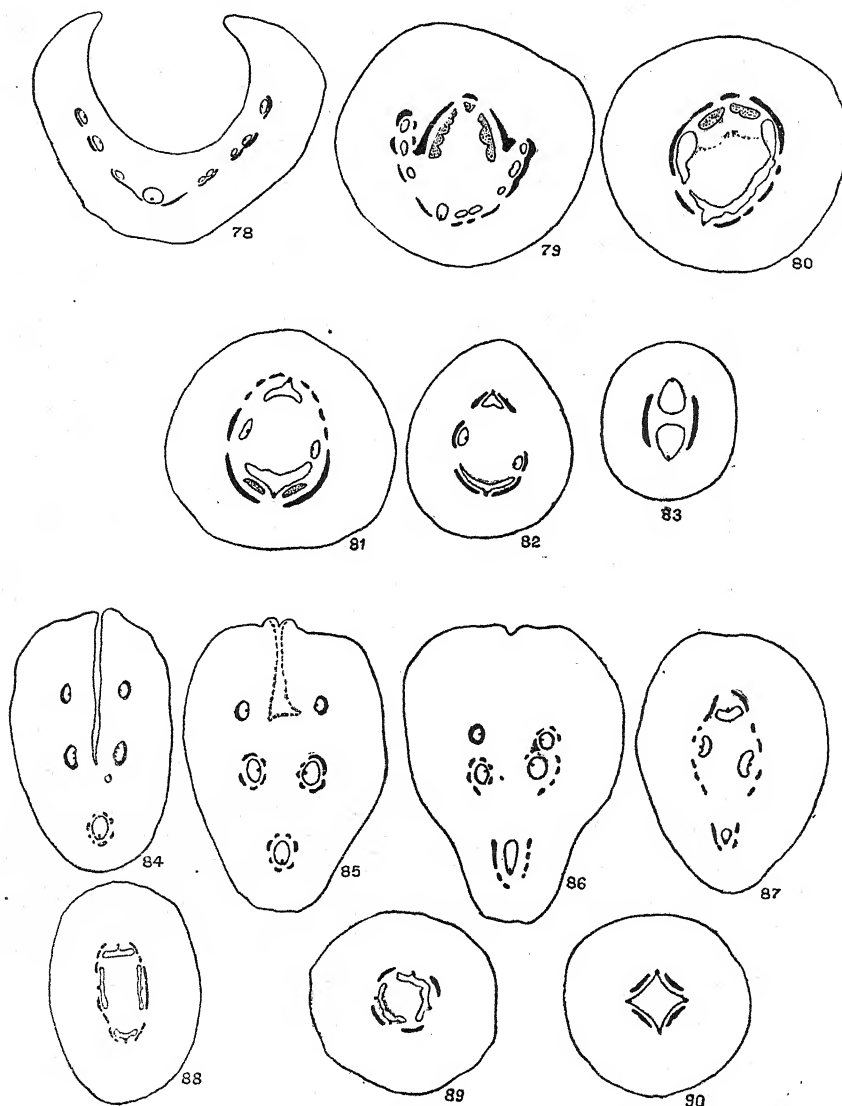
FIGS. 63-77. Figs. 63-69, simple type of Group II seedling, the cotyledon showing the structure of a single normal cotyledon and developing a diarch root-plate. Figs. 70-77, Group II seedling showing on the left delayed fusion of lateral and marginal bundles and on the right independence of the two.

lateral vascular system. This seems to be due in a measure to an increase in the relative importance of the marginal veins from the lamina, which, instead of uniting with the laterals at the distal end of the cotyledonary petiole, show a progressively delayed fusion until in many cases they only unite near the apex of the hypocotyl. This delay in fusion has inevitably

led to the entry into the petiole of smaller veins of secondary rank which normally would fuse with the marginal or lateral veins within the cotyledonary lamina. There is little doubt that the increase in the complexity of the petiolar vascular supply is to be correlated, in part at least, with the increase in the area of cross-section of that organ, this having become U- or C-shaped with the concavity on the adaxial side. One seedling of this type shows a curious anomaly in the arrangement of its petiolar vascular system, a presumably lateral strand lying in the same plane as, and immediately ventral to, the median strand. It retains this position throughout (Figs. 53 and 58-62), and the second pole of the diarch hypocotyl is produced by a concentration of the more distal lateral strands upon it, thus contrasting with the usual condition in which the concentration is bilateral and away from the middle line rather than towards it. There is also a further peculiarity in that the extreme marginal strand of one side is locally pseudoconcentric in character owing to the xylem being at that point completely surrounded by phloem (Fig. 59). From the stage in which the fusion of the lateral and marginal strands only occurs at the base of the petiole, further progress is inaugurated by this union being delayed until their entry into the hypocotyl. This is followed by the two becoming entirely independent (Figs. 81-83), and in one case the two strands of one side show delayed fusion, whilst on the other the marginal constituents alone take part in the formation of a root pole (Figs. 73-75). In such cases the strands representing the laminar laterals traverse the greater part of the hypocotyl, but ultimately their protoxylem disappears and their identity is lost in the xylem complex associated with the production of the root whorl to which reference has already been made. As long as the protoxylem persists it retains an endarch position, the only exception noted in the eight cases of this type being one in which it became mesarch towards its lower end. In one seedling both laterals are independent and persistent, and each forms a root pole so that a tetrarch condition is once more established (Figs. 84-90).

The seedling in which this phenomenon occurred was very short and stout (Figs. 22 *a*, 22 *b*), but whether this had any bearing on the development of tetrarchy it is impossible to say. A condition of secondary triarchy would obviously arise in a similar way granted an asymmetrical independence and persistence of one of the lateral strands. The median strand of this particular plant is remarkable in assuming a pseudoconcentric structure at the base of the petiole, though this is not persistent (Figs. 84-86), and a similar feature also characterizes the two lateral and to a certain extent the marginal strands immediately before and subsequent to their entry into the hypocotyl (Figs. 85-86). There is a distinct similarity between these strands and the 'Zwischenstränge' recorded by Dodel (5) in *Phaseolus* spp. and by Compton (2) in *P. Hernandezii*, *Abrus precatorius*, and in

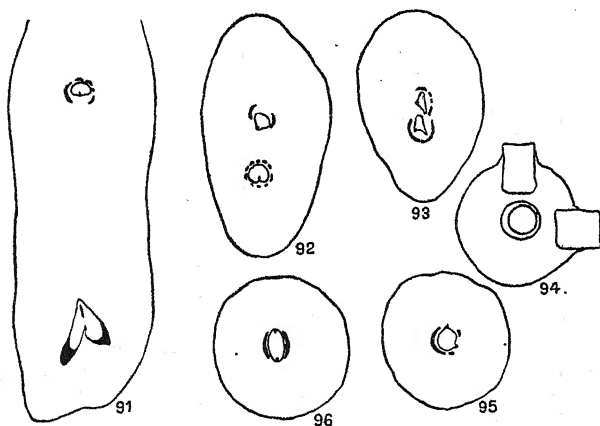
(3) *Helianthus annuus syncotyleus*, but the two do not seem exactly homologous, since the latter appear to be primarily hypocotyledonary in character and, although they occasionally contribute a root pole, they only exception-



FIGS. 78-90. Figs. 78-83, Group II seedling showing a further advance on that shown in Figs. 70-77, but still producing only a diarch root. Figs. 84-90, Group II seedling showing a still further advance leading to tetrachy in the root.

ally constitute a part of the laminar vascular supply, whilst in *Impatiens* the additional vascular complexity of the hypocotyl is the result of the modification and increase in importance of certain of the chief laminar strands.

A subsidiary line of evolution is exhibited by three seedlings in which a very complete cotyledonary tube is formed. Two of these show no special departure from the group as a whole in their vascular arrangements, but the third specimen is unique. In it there is no line of demarcation between cotyledonary petiole and hypocotyl, and the former consists of a more or less peltate basin-shaped structure, the floor of which is thrown into a number of wrinkles surrounding what looks like a small median pore (Figs. 7 *a*, 7 *b*). The vascular supply consists of the usual median, lateral, and marginal strands, but the two latter unite well inside the lamina, the resultant compound strands also fusing and becoming exarch very rapidly (Fig. 91). As a result the vascular system consists, immediately below the lamina, of two



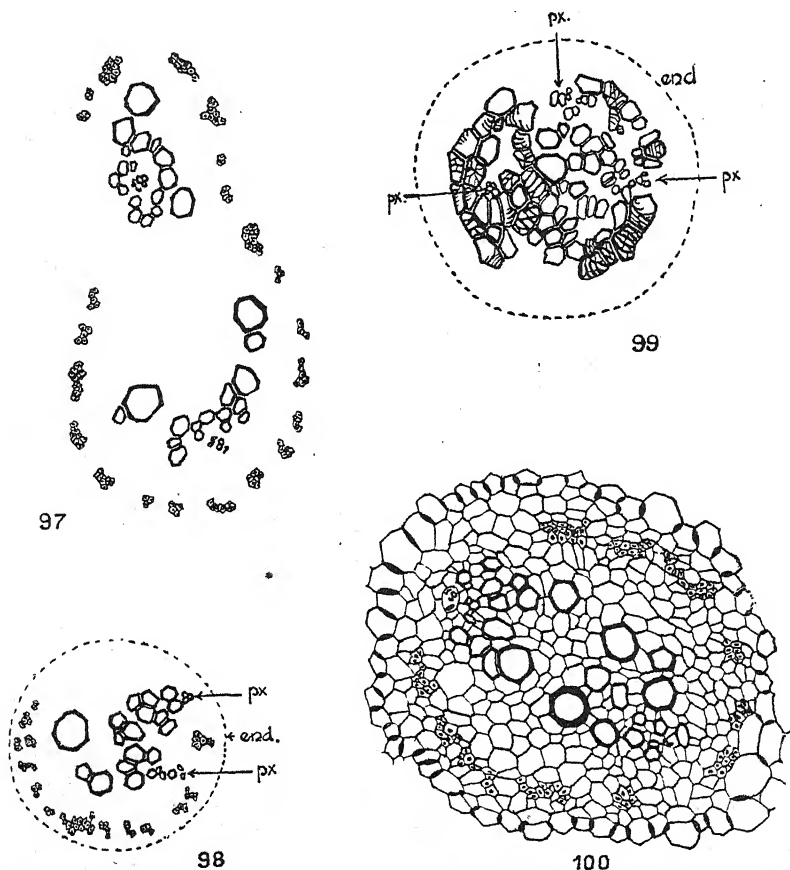
FIGS. 91-96. Anomalous Group II seedling with peltate cotyledon (Fig. 7), showing abnormal bundle behaviour.

bundles, the one derived from the laterals showing for a time the curious pseudoconcentric structure to which reference has been made in the other seedlings (Fig. 92). During their passage down the hypocotyl their behaviour is somewhat anomalous, that from the median strand remaining endarch, and turning outwards through  $90^\circ$ , whilst its exarch fellow also turns outwards through  $90^\circ$  to the same side (Fig. 93). The latter then swings back through  $180^\circ$ , whilst the endarch bundle becomes mesarch and finally exarch and in so doing comes to occupy its normal position. The result is that the two lateral roots representing the root whorl lie at right angles to each other (Figs. 94, 95), and it is not until these have been given off that the normal diarch plate is evident. At no stage is there any evidence, apart from the pore in the floor of the lamina, of the median cavity which the epicotyl would occupy. This may be due either to occlusion by the ingrowth of the walls of the tube, or possibly to the suppression of the petiole, a view which is supported by the early union of



the lateral strands and by the absence of any line of demarcation between cotyledon and petiole.

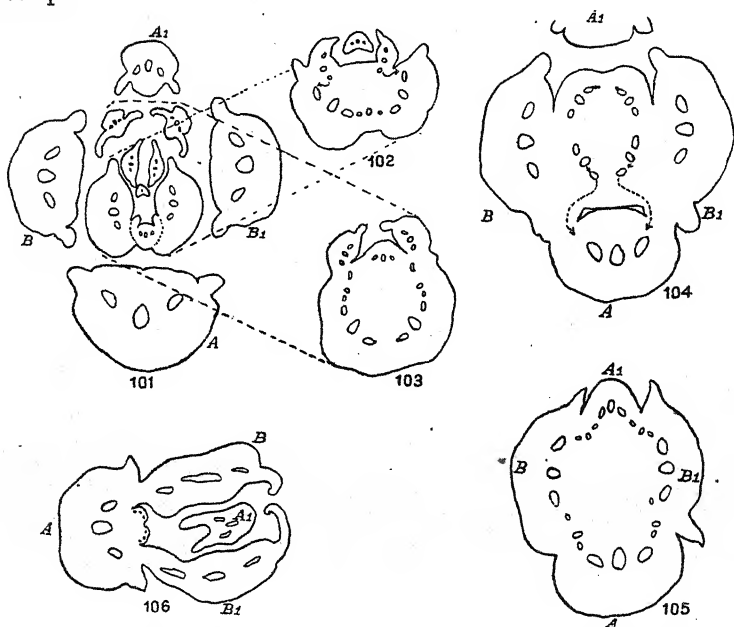
The modification of the epicotyl is along the same general lines as those indicated in the first group, but in the series now under consideration there is, with two possible exceptions, no trace of a second leaf at the first node,



FIGS. 97-100. Figs. 97, 98 illustrate the details of xylem and phloem distribution of Figs. 93 and 95. Fig. 99, tracheidal hypocotyledonary complex developed prior to the giving off of the basal root whorl from a syncotyl. Fig. 100, typical diarch plate in a Group II seedling.

though the second node is normal and bears two leaves. Where the seedlings are old enough the leaves of the third node are also two in number as in the first group. The evidence of epicotyledonary compression in the second group is thus as great as that found only in the extreme cases of the undoubted syncotyls. The exceptional cases referred to above were seedlings which were not discovered until the epicotyl had developed to a greater extent than that of the remainder of the series. Their salient features are illustrated in Figs. 101-106, and, as may be seen from these,

the arrangement of the leaves diverges very widely from the normal, so much indeed that it is impossible to correlate it at all closely with that of the remaining seedlings studied. Whether this divergence is an extreme expression of the effect of compression it is impossible, on such limited evidence, to say, but leaf *A* and, judging from the behaviour of its vascular strands, leaf *A* 1 in both seedlings arise at the first epicotyledonary node, and leaves *B* and *B* 1 at the second epicotyledonary node. Beyond these it is difficult to assign values, and little useful purpose would be served by the attempt.



FIGS. 101-106. Epicotyls of two Group II seedlings showing considerable modification of the leaf arrangements. Figs. 101-105 are from one seedling and Fig. 106 from a second.

Three views seem to be possible as to the origin of this group, and these may be briefly stated as follows:

(i) That the monocotylous condition is due to the suppression of one of the cotyledons, that is, the condition is heterocotylous in origin.

(ii) That the monocotylous condition is due to a degree of syncotyl of so extreme a character that there is no anatomical evidence of the double origin of the resultant cotyledon remaining.

(iii) That one of the cotyledons has retained its normal characteristics, whilst the other has become entirely leaf-like and is represented by the first epicotyledonary leaf.

The points in favour of the first hypothesis are based chiefly on the character of the cotyledon. The simplest member of the series exhibits

a cotylar anatomy which is indistinguishable in essentials from that of a single cotyledon of a normal dicotyl, and possesses, in common with the remaining members of the group, a single median axillary bud. Moreover, the group as a whole exhibits an admirably graded series of forms of progressively greater complexity, the final member of which is tetrarch owing to the increase in importance of the lateral and marginal strands respectively. If these seedlings were the ultimate products of a reduction series it would be reasonable to anticipate the development of a relatively stable type, whereas the reverse, as we have seen, is the case. A further point is that between the most extreme member of the undoubted syncotyls and the group under consideration there is a wide gap unbridged by any transitional forms whatever. It is recognized that negative evidence of this type is always liable to be shaken by subsequent discoveries, but it is fair to expect that some at least of the specimens studied would have furnished evidence of a double origin, since it is difficult to conceive of a cotyledonary union so intimate that no part of the seedling anatomy would reveal it, especially in view of the fact that syncotyl is not normal for the species.

The facts which tell against the above interpretation are three in number, namely:

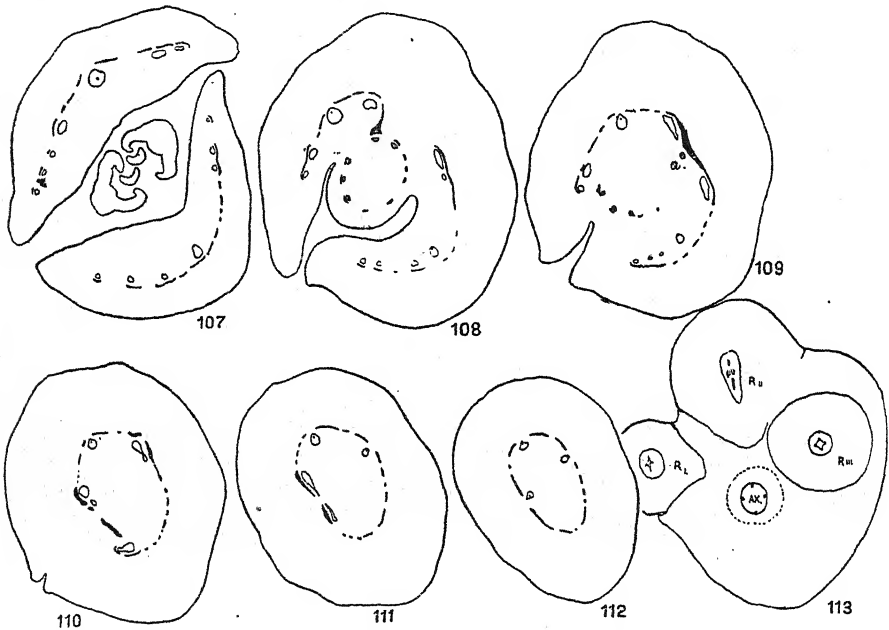
(i) The absence of material showing any trace of suppression of one of the cotyledons. (This, as negative evidence, is of course open to similar objections to those indicated in the previous paragraph.)

(ii) The position of the first epicotyledonary leaf.

(iii) The character of the modifications of the epicotyl.

The second and third of these points will be dealt with subsequently. With regard to the first, although a careful search was made and a number of seedlings exhibiting a difference in the size of the cotyledons were examined, no evidence of a convincing character was obtained. Where the discrepancy in the size of the cotyledons was not very pronounced the vascular structure was not affected, and where the cotyledon had remained very small this was due, with one exception, to traumatic causes. The injury responsible for the arrested growth as a rule involved a more or less complete severance of the median petiolar bundle, and often of one of the lateral bundles as well. The result was that, though the cotyledon became green, it retained its intraseminal form and size and was packed with starch grains owing to the interference with the channels of translocation. The one exceptional case, although not bearing directly on the origin of the group under discussion, is of sufficient interest to merit description. Of the two cotyledons, one was normal in size, whilst the other was small and was flexed downwards by the petiole (Figs. 8*a*, *b*, *c*). Serial sections revealed a curious anomaly in the vascular supply of the reduced cotyledon, the midrib consisting of a collateral bundle flanked, in the petiole, by three smaller bundles on either side (Fig. 107). At the apex of the hypocotyl

these bundles unite to form two groups which become widely separated and ultimately unite with the lateral bundles of the normal cotyledon, thus producing an asymmetrically triarch condition of the hypocotyl which is reflected in the development of three members only in the root whorl (Figs. 110-113). Below the point of origin of these roots the main root is tetrarch as in normal dicotyls. We have evidently to deal in this instance with a cotyledon in which the midrib is entirely lacking, its place being taken, in the lamina and petiole, by one of the laterals. With regard to the epicotyl this is chiefly remarkable from the fact that a whorl of three



FIGS. 107-113. Transverse sections of seedling with two unequal cotyledons (Fig. 8), showing suppression of the median double bundle in one cotyledon and the development of asymmetrical triarchy in the hypocotyl. R.i, R.ii, R.iii, lateral roots; AX., tetrarch axis.

leaves is produced at the second epicotyledonary node instead of the usual pair. The resultant modification in the grouping of the foliar traces in the epicotyl is indicated in Figs. 44-48. It is noteworthy that one of the compound epicotyledonary bundles (Fig. 109, *a*) is interposed between, and unites with, the approaching cotyledonary laterals on the same side, and that towards the base of the epicotyl the remaining strands form a shallow open gutter in the intercotyledonary plane.

The positive evidence in support of the origin of the second group of seedlings by syncotyl rests chiefly on the leaf position and the type of modification found in the epicotyl. The first epicotyledonary leaf stands in the position normally occupied by the second cotyledon, and if the cotyledon

is of dual origin this position represents what would be, in typical dicotyls, the intercotyledonary plane. If, on the other hand, the single cotyledon is heterocotylous in origin it is obvious that there has been an increase of ninety degrees in the angle of divergence between that structure and the first epicotyledonary leaf. There is no evidence in support of this change, though, in view of the fact that the epicotyledonary primordia originate much later than the cotyledons, it is conceivable that the modification in the latter may have induced an alteration in the orientation of the former.

The character of the epicotyledonary modifications themselves is wholly in favour of a syncotylous derivation, since the compression modifications typical of the more advanced syncotyls are reproduced faithfully in the seedlings comprising the second group.

It is worthy of note that in the monocotylous *Ranunculus Ficaria* and in all Monocotyledons proper<sup>1</sup> the seedling anatomy of which has been investigated, the insertion of the first epicotyledonary leaf is opposite that of the cotyledon. If this insertion is to be regarded as decisive in demonstrating a syncotylous origin of an apparently single cotyledon, then the Monocotyledons are undoubtedly of dicotylous origin. It must be remembered, however, that in embryonic tissues, such as are characteristic of an intraseminal stem apex, one is dealing, as Miss Sargent rightly emphasized, with extremely plastic material, and it is readily conceivable that the explanation of such an insertion is to be sought along physiological rather than phyletic lines. The third alternative need not be discussed at length. If the material investigated had been represented by apparent heterocotyls only, the conception might have been possible, although it would have involved a suppression of the vascular continuity between the modified cotyledon and the radicle. In the face of the evidence supplied by obvious syncotyls in which a gradual suppression of one leaf of the basal epicotyledonary pair can be demonstrated the idea becomes untenable.

It is of course realized that the evidence afforded by an anatomical study of teratological seedlings in a single species provides much too insecure a peg to hang a comprehensive theory upon, but one is perhaps justified in pointing out that, whichever view is adopted with regard to the origin of the second group of seedlings described, the result gives food for thought. If syncotily is invoked, then we have, in a species normally dicotylous, teratological syncotyls in which all trace of double origin is lost: if, on the other hand, a heterocotylous origin is regarded as more probable we are faced with the fact that syncotily and heterocotily are possible within the limits of a single species. A comparison of the cotyledonary anatomy of the second group of seedlings with that of *Ranunculus Ficaria* reveals

<sup>1</sup> E. g. *Albuca Nelsoni*, *Allium* spp., *Anemarrhena asphodeloides*, *Anthericum Liliago*, *Arisaema dracontium*, *Arthropodium cirrhatum*, *Arum maculatum*, *A. italicum*, *Chlorogalum pomeridianum*, *Fritillaria imperialis*, *Hyacinthus romanus*, *Triglochin palustre*, *Zygadenus elegans*.

one interesting point of similarity, this being the increase in the relative importance of the marginal strands in some of the specimens figured by Sterckx (15). Thus his Fig. 178 shows the marginal strands deferring their fusion with the median strand until well down the cotyledonary petiole, and Figs. 173 and 176 illustrate cases in which the strands are apparently independent throughout the petiole. A similar increase in the relative importance of the lateral strands is also characteristic of many monocotyledonous seedlings, although further complications are produced in many of these by the vascular system of the first epicotyledonary leaf contributing to that of the hypocotyl. Thus independent laterals characterize both *Arum maculatum* (14) and *A. italicum* (1), and Sargent (12) has described a number of species in the Scilleae (Liliaceae) in which all stages are shown, varying from a condition in which the contributions to the vascular symmetry of the radicle by the cotyledonary lateral strands are more or less capricious (e.g. *Galtonia candicans*, *Dipcadi serotinum*) to forms in which the lateral bundles of the cotyledon take a perfectly regular share in the formation of the root stele. 'The species *Hyacinthus romanus*, *Muscari atlanticum*, *M. armeniacum*, and *M. neglectum*, for example, form a series in which the lateral traces become more and more important until they supply a full half of the root stele.'

Special mention must be made of the condition characterizing *Anemarrhena asphodeloides* (10), in which extreme compression of the median and lateral bundles has resulted in their fusion to form two massive bundles situated one on either side of the sagittal plane of the seedling. The protoxylem of these bundles divides into three, the median portions each constituting a root pole situated in the intercotyledonary plane, whilst the others unite in pairs in the cotyledonary plane, so that a tetrarch root is formed. Sargent (12) regards the massive cotyledonary bundles as homologous with the 'double bundle' characterizing the cotyledon of such forms as *Allium*, and there is little doubt that this is true, though of a portion only, of each of the *Anemarrhena* bundles. Chauveaud (1) has shown, however, that the widely separated halves of the double bundle of *Allium* originate as a radial file of protoxylem elements flanked by primary phloem, and that the wide separation of the two xylem segments in the maturer condition of the cotyledon is due to the successive resorption first of the radial file of vessels (Chauveaud's 'vaisseaux alternes') and then of the intermediate vessels succeeding it on either side. The three stages are admirably illustrated in Chauveaud's figures (1, pp. 177-179, Figs. 20-25). A further point of interest in this connexion is provided by a comparison of Chauveaud's Fig. 25, illustrating the last remnants of the crushed and partly resorbed intermediate vessels in *Allium*, with Sargent's figure of a section across the cotyledon of *Anemarrhena* (10, Pl. XXXIII, Fig. 2). The latter exhibits evidence for the resorption of intermediate vessels lying

between the two xylem masses strikingly like that given by Chauveaud in *Allium*. There is little doubt that an examination of younger seedling material of *Anemarrhena* would reveal both 'alterne' and intermediate elements situated in the region separating the two divergent bundles characterizing the older cotyledon. If this interpretation of the structure of the *Anemarrhena* be the correct one, it follows that each xylem 'massif' really represents the fused constituents, not of a median bundle with a lateral on either flank, but of half a median bundle with both a lateral and a marginal strand on the distal side only. A certain amount of collateral support for this interpretation is provided by Chauveaud's description of the seedling anatomy of *Cordyline indivisa* (1, pp. 421-425). This seedling has a tetrarch root, but in the young state only one xylem is differentiated in the hypocotyl, the remainder of the root poles being represented by their phloem only. The phloem groups coalesce to form two larger composite groups flanking the persistent xylem, which becomes the cotyledonary midrib. It is evident that in *C. indivisa* there is an *Anemarrhena* condition in which the lateral and marginal xylem elements are not differentiated in the hypocotyl till a relatively late stage. There is thus in *Cordyline indivisa*, and probably in *Anemarrhena asphodeloides* also, a close parallel to the condition found in the tetrarch seedling of the second group described in this paper, the only essential difference being that consequent on the crowding together of the lateral and marginal and median strands in the two monocotyledonous species. The evidence for regarding the vascular system of *Anemarrhena* in this way is admittedly somewhat sketchy and incomplete, but it serves to emphasize the urgent need for a reinvestigation of the very young stages of the seedling anatomy of Monocotyledons, a task which it is hoped to proceed with during the coming summer.

#### SUMMARY.

1. The bundle-system of each normal cotyledon of *Impatiens Roylei* consists of a median double bundle with a lateral bundle on either flank, these producing tetrarch symmetry in the hypocotyl and root.
2. The young epicotyl produces paired leaves at the first two nodes and whorls of three members at subsequent nodes. The vascular anatomy of these leaf-systems and their interrelationships is described.
3. The atypical seedlings fall into two main groups, the first of which is undoubtedly syncotylous in origin.
4. The seedlings of this first group show a graded series in which the syncotyly becomes progressively more intimate, this resulting in the suppression of the lateral bundles, and consequently of the root pole, on the symphysis side.
5. The effect of syncotyly on the epicotyl is shown in the reduction and



ultimate disappearance of the first epicotyledonary leaf on the symphysis side, and possibly, in extreme cases, the modification of the leaves at the third node. The leaves of the second node are usually not affected.

6. The seedlings of the second group may be either syncotylous or heterocotylous. In favour of the first alternative there is the position of the first epicotyledonary leaf and the character of the epicotyledonary modifications as a whole: in favour of the second alternative is the cotyledonary morphology, its anatomy, and the possession of a single axillary bud.

7. The seedlings of the second group show certain anatomical tendencies due to the increase in the relative importance of the lateral and marginal strands, which are paralleled to some extent by the lateral strands in many seedling Monocotyledons.

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# The Growth of Lemna Plants in Mineral Solutions and in their Natural Medium.

BY

W. B. BOTTOMLEY, M.A., PH.D.

*Professor of Botany at King's College, London.*

A CONSIDERABLE amount of research has been carried out by the author during the past few years, on the growth of water plants, chiefly *Lemna minor*, in culture solutions. The results of some of this research have already been published,<sup>1</sup> and the work indicates that these plants are unable to maintain their normal health and vigour in solutions containing only mineral salts, while when supplied with small quantities of organic substances they rapidly increase in number, remaining at the same time perfectly healthy. Further experiments, which have recently been published,<sup>2</sup> on the beneficial effect of nucleic acid derivatives, which the author has found can be extracted from raw peat,<sup>3</sup> and of the products of nitrogen-fixing organisms, show that it is the *organic matter* of these additions which brings about the increase in growth, since the ash of the substances has no effect on either the rate of growth or the health of the plants.

## COMPARISON OF DIFFERENT NUTRIENT SOLUTIONS.

There was the possibility, however, that the failure of these plants to maintain a rapid rate of multiplication and their normal healthy appearance in the control series of the experiments already recorded might be due to an unsuitable combination or proportion of the mineral substances in the nutrient solution employed. Throughout the experiments the solution used was that advocated by Detmer-Moor,<sup>4</sup> containing—

Potassium nitrate, 7 grm.  
Di-potassium phosphate, 1.5 grm.  
Magnesium sulphate, 1.5 grm.  
Sodium chloride, 1.5 grm.  
Ferric chloride—a few drops.  
Calcium sulphate in excess (5 grm. were used).  
Distilled water, 3,000 c.c.

<sup>1</sup> Bottomley, W. B.: Proc. Roy. Soc., B, vol. lxxxix, 1917, pp. 481-507.

<sup>2</sup> Ibid., vol. xci, 1919, pp. 83-95.

<sup>3</sup> Ibid., vol. xc, 1917, pp. 39-44.

<sup>4</sup> Detmer-Moor: Practical Plant Physiology, London, 1898, p. 85.

It was therefore decided to carry out a series of experiments with Knop's nutrient solution, containing—

Potassium nitrate, 1 grm.  
 Potassium biphosphate, 1 grm.  
 Magnesium sulphate, 1 grm.  
 Calcium nitrate, 3 grm.  
 Ferric chloride—a few drops.  
 Distilled water, 6,000 c.c.

The concentration of nutrient salts in this solution reaches about 1,000 parts per million, as compared with 5,500 parts per million in Detmer's solution.

Fifteen dishes were arranged towards the end of September in three series of five dishes each: those of Series I, numbered from 1 to 5, each containing 150 c.c. of Detmer's solution; those of Series II, numbered from 6 to 10, each containing 150 c.c. of Knop's solution; and those of Series III, numbered from 11 to 15, each containing 150 c.c. of Knop's solution plus the water extract of 1 grm. of bacterized peat per 1,000 c.c. of solution. A fourth series of five dishes, to which reference will be made later, was also inserted here, for the purpose of avoiding the duplication of the control series which would be necessary in a separate experiment. Twenty plants of *Lemna minor* were placed in each dish, and 300 similar plants counted out for an estimation of their dry weight, which was found to be 12.5 mg. for 100 plants. The same precautions for excluding light from bottom and sides of dishes, and for protecting them from dust, were observed as in the preceding experiments, and the liquids were renewed twice weekly, the greatest care being taken to prevent the growth of green Algae, which would supply organic matter to the solutions. Once a week the plants were counted, and they were halved at the weekly counting when necessary, one half being discarded. This did not happen, however, until the sixth week, for since the experiment did not end until about the middle of November, and was carried out in an unheated greenhouse, the rate of growth was extremely slow. The figures obtained for the eight weeks during which the experiment lasted are shown in the table below.

TABLE I.

Series.	Dish No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.
I. Detmer's Solution	1	34	64	132	200	246	304	334	420
	2	40	80	164	222	273	300	348	432
	3	38	76	156	202	261	324	382	434
	4	39	72	160	208	244	302	366	440
	5	40	82	171	201	239	330	378	456
	Mean	38.2	74.8	156.6	206.6	252.2	312.2	361.9	436.4

Series.	Dish No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.
II. Knop's Solution	6	28	60	93	159	202	256	268	286
	7	33	62	102	161	200	244	282	316
	8	27	62	90	158	201	256	264	308
	9	32	61	93	152	209	240	302	354
	10	32	66	101	166	230	278	328	372
	Mean	30.4	62.2	95.8	159.2	208.4	254.8	288.8	327.2
III. Knop's Solution + Bacterized Peat	11	36	60	118	207	286	422	562	740
	12	35	73	137	247	354	456	590	754
	13	33	69	127	220	322	408	570	730
	14	38	74	125	237	310	470	684	848
	15	34	68	123	225	310	428	604	804
	Mean	35.2	68.8	126.0	227.2	316.4	436.8	602.0	775.2

The dry weights of the half-sets which were discarded in the sixth week were estimated, and a similar determination was made at the conclusion of the experiment, the following comparison for the three series being obtained:

TABLE II.

	6th week.			8th week.		
	Average No. of Plants.	Average weight of Plants.	Calculated weight of 100 Plants.	Average No. of Plants.	Average weight of Plants.	Calculated weight of 100 Plants.
		mg.	mg.		mg.	mg.
Series I.	312	33.1	10.6	436	36.0	8.2
Series II.	255	27.4	10.7	327	29.2	8.9
Series III.	437	75.2	17.2	775	124.0	17.3

Owing to the advanced season of the year, the rate of growth in all the series was extremely slow as compared with that obtained in other experiments, recorded in the previous work,<sup>1</sup> which were carried out during the growing season under conditions of fairly high temperature and continuous sunlight during the greater part of the day. However, the experiment demonstrates quite clearly that Knop's solution is no more able than is Detmer's to supply the plants with all that they require to maintain their normal health. In the former solution the weight of 100 plants depreciated from 12.5 mg. at the beginning to 8.9 mg. at the end, while in the series supplied with organic substance the plants showed not only a more rapid rate of multiplication, but also an increase in individual size, the weight of 100 plants at the end of the experiment reaching in this series 17.3 mg.

#### EXPERIMENT WITH *LEMNA MAJOR*.

A similar experiment had also been arranged and carried out with *Lemna major* a little earlier in the season, being started at the end of August. A set of twenty dishes was divided into four series of five dishes each. Those of Series I, numbered from 1 to 5, each contained 150 c.c. of

<sup>1</sup> Bottomley, W. B.: Proc. Roy. Soc., B, vol. lxxxix, 1917, pp. 481-507.

Detmer's solution, and those of Series II, numbered from 6 to 10, a similar quantity of the same solution together with the extract of 1 grm. of bacterized peat in every 1,000 c.c. Series III and IV were precisely similar to Series I and II respectively, except that Knop's solution was used instead of Detmer's. Ten plants of *Lemna major* were put into each dish, and fairly young plants were chosen instead of quite full-grown ones, in order that a more uniform selection as to size might be made. The dry weight of three separate hundreds of similar plants was estimated, and found to be 36.6, 36.0, and 34.8 mg. respectively, giving an average of 35.8 mg. per 100 plants. The dishes were treated precisely as before, their solutions being changed twice weekly and the plants counted once weekly. They did not multiply nearly so rapidly as did the *Lemna minor*, and so there was no necessity to halve the contents of the dishes, though the experiment lasted for five weeks. The number of plants obtained each week during the course of the experiment are shown below.

TABLE III.

Series.	Dish No.	1st week.	2nd week.	3rd week.	4th week.	5th week.
I. Detmer's Solution	1	27	33	44	57	62
	2	27	34	44	53	57
	3	18	24	28	39	43
	4	24	25	34	40	45
	5	22	32	39	41	41
	Mean	23.6	29.6	37.8	46.0	49.6
II. Detmer's Solution + Bacterized Peat	6	31	50	72	115	161
	7	31	45	62	102	126
	8	30	48	75	118	154
	9	29	47	70	110	150
	10	27	43	60	100	129
	Mean	29.6	46.6	67.8	109.0	144.0
III. Knop's Solution	11	18	23	25	29	31
	12	25	26	28	31	31
	13	22	23	28	29	29
	14	18	21	27	29	29
	15	22	24	27	28	30
	Mean	21.0	23.4	27.0	29.2	30.0
IV. Knop's Solution + Bacterized Peat	16	31	45	75	96	162
	17	29	40	53	77	112
	18	29	45	70	82	130
	19	28	35	49	65	108
	20	27	44	71	96	151
	Mean	28.8	41.8	63.6	83.2	132.6

At the conclusion of the experiment the dry weight of the contents of three of the dishes in each series was estimated, and from the figures obtained the weight of 100 plants was calculated. The results were as follows:

Weight of 100 plants at beginning of experiment	.	= 35.8 mg.
"	"	in Series I at end of experiment = 30.8 mg.
"	"	" Series II " " = 52.8 mg.
"	"	" Series III " " = 29.4 mg.
"	"	" Series IV " " = 50.5 mg.

A striking feature of the experiment was the fact that although young plants were chosen at the beginning, those in mineral salts only never increased in size, while those supplied with organic matter rapidly became full grown; and although the energy of these plants was being expended in multiplication to a far greater extent than in the control series, yet the individual new plants which were budded off rapidly reached the mature condition. The plants in mineral nutrients only seemed to be quite unable from the beginning to attain their normal size, and began to multiply in their immature state. The figures given indicate quite clearly that *Lemna major*, in common with *Lemna minor*, cannot maintain its normal health and vigour in solutions containing only inorganic nutrients, and although Detmer's solution favoured the multiplication of these plants rather better than that of Knop, yet there was no appreciable difference in the size and health of the plants in the two series. The variation was one of number only, and the weights of 100 plants in the two series were practically similar at the end of the experiment. Brenchley<sup>1</sup> has found that the rate of growth of wheat and barley in water culture increases with the concentration of the nutrient solution, and the more rapid rate of multiplication in the Detmer's solution may have been due to the greater concentration of nutrients supplied in this as compared with Knop's solution, but in both media the plants completely failed to maintain their health.

#### GROWTH OF *LEMNA MINOR* IN POND WATER.

Such mineral solutions as those used in the above experiments, however, bear no comparison to the natural habitat of *Lemna* plants, and in the course of the work no comparison had hitherto been made of the growth obtained in mineral nutrients and in the water of the pond in which the plants were originally growing. Accordingly, in the experiment recorded above, started towards the middle of September, an extra set of five dishes was prepared, called Series IV and numbered from 15 to 20. These dishes each contained 150 c.c. of pond water, and the plants were treated precisely as in the other series, being counted once weekly, and having the medium renewed twice weekly. For each renewal of this series fresh water was obtained from the pond, and filtered from suspended matter.

At the end of the sixth week the plants in this, as in the other series, were halved, and the dry weight of one half taken. This was repeated at

<sup>1</sup> Brenchley, W. E.: *Annals of Botany*, vol. xxx, No. cxvii, 1916, pp. 77-90.

the end of the eighth week. The figures obtained for the complete sets in Detmer's solution and in pond water each week are shown in the table below.

TABLE IV.

Number of Plants.	Series IV. Pond Water.						Series I. Detmer's Solution.					
	Dish Number.						Dish Number.					
	16	17	18	19	20	Mean.	1	2	3	4	5	Mean.
At beginning	20	20	20	20	20	20.0	20	20	20	20	20	20.0
1st week	30	30	31	27	32	30.0	34	40	38	39	40	38.2
2nd "	52	55	53	49	57	53.2	64	80	76	72	82	74.8
3rd "	63	72	75	69	81	72.0	132	164	156	160	171	156.6
4th "	92	86	89	89	91	89.4	200	222	202	208	201	206.6
5th "	94	110	93	109	107	102.6	246	273	261	244	239	252.2
6th "	102	116	104	114	110	109.2	304	300	324	302	330	312.0
7th "	142	122	112	126	122	124.8	334	348	382	366	378	361.9
8th "	168	156	120	160	144	149.6	420	432	434	440	456	436.4

*Weight of Plants in mg.*

6th week	13.9	15.8	14.4	15.4	15.0	14.9	31.8	31.9	35.4	30.6	35.6	33.1
8th "	22.4	20.8	18.4	22.0	19.2	20.6	32.0	35.4	37.3	36.9	38.3	36.0

A comparison of the mean weights and numbers of the plants in the two series for the sixth and eighth weeks gives the following figures:

TABLE V.

	Series IV. Pond Water.			Series I. Detmer's Solution.		
	Average No. of Plants.	Average weight of Plants.	Calculated weight of 100 Plants.	Average No. of Plants.	Average weight of Plants.	Calculated weight of 100 Plants.
		mg.	mg.		mg.	mg.
6th week	109.2	14.9	13.6	312.0	33.1	10.6
8th "	149.6	20.6	13.7	436.4	36.0	8.2

The dry weight of 100 plants at the beginning of the experiment was 12.5 mg., so that although the plants in pond water did not multiply so rapidly as did those in Detmer's solution, they maintained and even increased upon their original size. They also retained their normal green colour throughout and were perfectly healthy at the end of the experiment, while those in Detmer's solution diminished in size and became very unhealthy in appearance.

It is thus evident that the pond water contained certain essentials for normal metabolism which were lacking in Detmer's solution, though the rate of growth in the former medium appeared to be limited by the insufficient quantities of mineral nutrients, which were so abundant in the latter.

An analysis of four samples of the water taken from the pond during four successive weeks showed an average content of 1,577 parts per million of total dissolved solids, of which 1,089 parts were inorganic and 488 parts consisted of organic matter. Further examination showed that the water



contained small amounts of nitrates and of phosphates which could serve as nutrients. In spite of this restriction of food supplies, the plants remained healthy in appearance and slightly increased their original size, this increase being probably due to the lack of competition normally met with in their natural situation, combined with the more uniform conditions prevailing during the experiment. It is therefore presumably the presence of the *organic* matter which enables the plants to maintain their normal health, while the lack of food materials acts as a limiting factor restricting their rate of multiplication. The absence of this organic matter from the solutions probably explains the difficulty experienced by Darwin and Acton<sup>1</sup> in growing water plants in culture solutions.

#### SUMMARY AND CONCLUSIONS.

The experiments here recorded emphasize the necessity for organic matter for the optimum growth and development of *Lemna minor*. Previous work on the part of the author has shown that these plants are unable to grow healthily for any length of time in Detmer's nutrient solution, while the addition of organic matter both accelerated the growth and promoted the health of the plants.

Experiments carried out with Knop's solution demonstrate that this is no more capable than is Detmer's medium of maintaining the plants in health, while the former solution together with organic substance enables the plants to multiply more rapidly and retain a healthy appearance.

The failure of the nutrient solution previously employed to satisfy the needs of the plants is therefore not due to an unsuitable combination of materials, since the widely used Knop's solution is equally inefficient.

Plants of *Lemna major* showed a similar requirement for organic material. Young plants used at the beginning of the experiment, when grown in mineral nutrients only, even failed to attain the adult size, but began to multiply in the immature condition, while the addition of organic substance had the effect of enabling the plants to rapidly become full grown, at the same time showing an increased rate of multiplication.

The growth of plants of *Lemna minor* in mineral solutions was compared with that in the water of the pond in which they were originally growing, with the result that though in the latter medium the plants did not multiply so rapidly, they retained and even slightly increased upon their original size and remained quite healthy.

Analysis of the pond water showed an average content of 1,577 parts per million of total dissolved solids, of which 488 parts were organic. The inorganic constituents included very small quantities of nitrates and phosphates.

<sup>1</sup> Darwin and Acton: Practical Physiology of Plants, Cambridge, 1901, pp. 61-3.

The experiments recorded show that *Lemna minor* and *Lemna major* are not able to grow normally in solutions containing inorganic materials only, but that the addition of certain organic substances to these solutions permits a rapid and healthy growth. These organic substances essential to the metabolism of the plants are to be found in the water of the ponds in which they normally grow, and maintain the plants in health, although lack of quantities of nitrates and phosphates in these conditions acts as a limiting factor retarding their rate of multiplication.

# The Effect of Organic Matter on the Growth of various Water Plants in Culture Solution.

BY

W. B. BOTTOMLEY, M.A., PH.D.,

*Professor of Botany at King's College, London.*

With Plate XVII.

THE importance of organic manures in agricultural operations is a well-recognized fact, and a considerable amount of evidence has been accumulated by the author in support of the view that such organic manures are of direct importance in the nutrition of the plant, quite apart from their indirect effect in altering and improving the physical condition of the soil and in providing food for soil bacteria. It has already been shown that plants of *Lemna minor* will not grow and flourish normally in solutions containing mineral nutrients only, and that the addition to such nutrient solutions of small quantities of organic substances obtained from decomposing vegetable matter,<sup>1</sup> from cultures of nitrogen-fixing organisms, and from nucleic acid and its derivatives<sup>2</sup> which the author has found can be extracted from raw peat,<sup>3</sup> enable the plants to multiply rapidly and retain their healthy appearance.

It has also been shown<sup>4</sup> that the failure of a pure inorganic solution to support normal growth in these plants is not due to an unsuitable balance of nutrient materials, for similar results were obtained with more than one solution in common use among experimenters with water cultures; while trials carried out with water of the pond in which the plants were growing showed that in this medium they maintained their normal health and vigour, although their rate of multiplication was retarded as compared with that in the artificial nutrient solution, presumably owing to the lack of any large quantities of the essential inorganic materials in the pond water. The organic matter, which this water contained to the amount of nearly five hundred parts per million, enabled the plants to grow healthily throughout.

<sup>1</sup> Bottomley, W. B.: Proc. Roy. Soc., B., vol. lxxxix, pp. 481-507 (1917).

<sup>2</sup> Ibid., vol. xci, pp. 83-95 (1919).

<sup>3</sup> Ibid., vol. xc, pp. 39-44 (1917).

<sup>4</sup> Ibid.: Annals of Botany, vol. xxxiv, No. cxxxv (1920).

The question arose as to whether this necessity for organic matter is a peculiarity of water plants as distinct from land plants, and whether, among water plants, *Lemna* stands alone in such requirement. A preliminary experiment has already been reported with *Lemna major*,<sup>1</sup> which indicated that the behaviour of this plant corresponds with that of *Lemna minor* in that it fails to grow normally in a mineral solution, while the addition of water-soluble organic matter from bacterized peat enables it to maintain its health and increase its rate of multiplication. This experiment, however, was essentially of a preliminary nature, and was carried out towards the end of the growing season when no rapid multiplication could be expected.

#### EXPERIMENTS WITH *LEMNA MAJOR*.

A more extensive trial with *Lemna major* was made about the middle of May, to determine whether this plant would still fail to grow well in mineral nutrients even during the good growing season, and also to discover whether organic substances other than bacterized peat could supply the deficiency in this case as in the case of *Lemna minor*.

Four series of five dishes each were arranged, each containing 150 c.c. of Detmer's solution. The dishes of Series I, numbered from 1 to 5, served as controls, while the following additions were made to the other dishes: Series II, numbered from 6 to 10, the crude nucleic acid derivatives from one gramme of raw peat in every 500 c.c. of solution; Series III, numbered from 11 to 15, one gramme of autoclaved *Azotobacter* growth in every 1,000 c.c.; and Series IV, numbered from 16 to 20, the water extract of one gramme of bacterized peat in every 500 c.c. These various substances were obtained in precisely the same way as described in the previous publications.

Ten plants of *Lemna major* were counted out into each dish, and care was taken to select very young plants, instead of full-grown ones, in order that their size might be more uniform. Three hundred similar plants were taken at the same time and well washed for an estimation of their dry weight, which was found to be 24.2 mg. for 100 plants.

The dishes were surrounded up to the level of the liquids with paper which was black on the side towards the dish and white towards the outside, in order to exclude light from the bottom and sides. The whole set was covered with a large sheet of glass supported a little above the top of the dishes, in order to prevent the access of dust as much as possible. The solutions were changed twice weekly, and the plants counted once weekly, care being taken to exclude green algae as much as possible. The figures obtained in the first three weeks of the experiment are shown in the table below:

<sup>1</sup> Bottomley, W. B.: *Annals of Botany*, vol. xxxiv, No. cxxxv (1920).

TABLE I.

Series.	Dish No.	1st week.	2nd week.	3rd week.
I. Detmer's solution	1	18	25	30
	2	18	22	28
	3	18	23	26
	4	18	23	28
	5	18	24	30
	Mean	18	23.4	28.4
II. Detmer's solution + crude nucleic acid derivatives	6	26	57	179
	7	24	51	163
	8	26	58	189
	9	23	49	156
	10	24	57	184
	Mean	24.6	54.4	174.2
III. Detmer's solution + autoclaved <i>Azoto-</i> <i>bacter</i>	11	20	33	61
	12	19	27	51
	13	19	28	54
	14	20	32	59
	15	20	29	49
	Mean	19.6	29.8	54.8
IV. Detmer's solution + bacterized peat	16	24	65	250
	17	23	71	272
	18	22	67	258
	19	22	63	246
	20	25	71	250
	Mean	23.2	67.4	255.2

At the end of three weeks the numbers and also the appearance of the individual plants were so striking as to render the continuation of the whole series unnecessary, so on account of pressure of other work the experiment as a whole was brought to a close, although the dishes of Series I and IV were halved, one half of each dish being used for an estimation of the dry weight of the plants, and the other half of these two series being continued for a further period of three weeks. At the end of this time the average number of plants in Series I, resulting from the original 10, was 72, and that in Series IV 4.704.

At the time when the main part of the experiment ceased, three weeks after its commencement, the plants in Series I were extremely small and yellow and unhealthy in appearance. The plants, though not full-grown at the beginning of the experiment, had never increased in size, but had budded slowly, the new plants formed being successively smaller than the originals—so much so that it was quite easy at the end to pick out the original ten which were placed in the dishes, on account of their larger size. The plants in Series II, III, and IV at the end of the three weeks were all large,

green, and healthy in appearance. A comparison of the dry weights of the plants at this period gave the following figures :

100 plants at the beginning in all series weighed	24.8 mg.
100 plants after three weeks in Series I	20.4 "
" " " II	44.8 "
" " " III	47.2 "
" " " IV	46.5 "

These figures clearly indicate that *Lemna major* is not able to maintain a good healthy growth in solutions containing only mineral nutrients, and that the addition of the various organic substances supplied corrected the deficiency and enabled the plants to grow more luxuriantly and preserve a thoroughly healthy appearance. The varying effect of the different substances added is chiefly shown in their influence on the rate of multiplication, for the weights of the plants in all the series containing organic matter were approximately equal, and all appeared equally healthy.

It was pointed out that the plants used in these experiments were immature at the beginning, so a further trial was made in July, when a fresh supply of these plants came to hand, to determine whether fully-grown plants could remain healthy in inorganic nutrients only.

Two series, each consisting of five dishes, were arranged, those of Series I, numbered from 1 to 5, each containing 200 c.c. of Detmer's solution, and those of Series II, numbered from 6 to 10, containing a similar quantity of the same solution, which also contained the water extract of 1 grm. of bacterized peat in every 500 c.c. Ten full-grown plants of *Lemna major* were placed in each dish, and three hundred similar plants used for an estimation of their dry weight. The dishes were treated precisely as before, for four weeks, and the following figures were obtained :

TABLE II.

Series.	Dish No.	1st week.	2nd week.	3rd week.	4th week.
I. Detmer's solution	1	16	20	21	30
	2	13	18	20	29
	3	13	17	20	26
	4	12	16	19	25
	5	17	18	21	28
	Mean	13.8	17.8	20.2	27.6
II. Detmer's solution + bacterized peat	6	24	48	99	166
	7	28	53	113	197
	8	26	50	104	173
	9	25	49	92	168
	10	24	51	103	192
	Mean	25.4	50.2	102.2	177.2

The plants in dishes 2, 5, 6, and 8, which were nearest to the mean, were pressed and mounted as a permanent record, and photographs of these

pressed plants are shown in Pl. XVII, Figs. 1 and 2. The plants in the remaining dishes were used for determinations of the dry weight of the plants, with the following results :

100 plants at the beginning of the experiment in both series

weighed 38.4 mg.

100 plants at the fourth week in Series I       "   22.0 "

"   II   "   51.9 "

Even though these plants were fully grown to begin with, their offspring were clearly unable to attain their normal size in the mineral nutrients, while those supplied with organic matter appreciably increased upon the weight of the original plants. The yellowish colour of the control plants was in such marked contrast to the rich green of those in the organic substance that it was feared that the nutrient solution might be lacking in sufficient quantity of iron. An extra quantity of iron was therefore added to the control series, but it had absolutely no effect on either colour or size of the plants.

It is clear from these experiments that certain organic substances are quite as necessary for *Lemna major* as for *Lemna minor*, and that without such materials the plants rapidly become unhealthy, their individual weight being diminished and their rate of multiplication retarded.

#### EXPERIMENTS WITH *SALVINIA NATANS*.

Somewhat late in the season, towards the end of August, some experiments were commenced with *Salvinia natans*. A set of ten dishes was prepared, divided into two series of five dishes each. Those of Series I, numbered from 1 to 5, each contained 250 c.c. of Detmer's solution, and those of Series II, numbered from 6 to 10, contained a similar quantity of this solution with the addition of the extract from one gramme of bacterized peat per 1,000 c.c. Into each of the dishes were put small sprigs of *Salvinia natans*. It was a little more difficult than in the case of *Lemna* to select absolutely uniform samples of this plant, but the portions in the ten dishes were chosen as equally as possible, each dish receiving two sprigs each consisting of three leaves and a bud, and two sprigs of two leaves and a bud each. Each dish therefore contained ten full-grown leaves and four buds. Four similar sets were chosen at the same time and their dry weights estimated. The figures obtained were 10.4, 10.0, 10.0, and 10.2 mg. respectively, giving an average of 10.15 mg. as the original dry weight of each set. The under surface and the roots of these plants when received were closely covered with a mass of blue-green algae, which was carefully removed by washing before the experiment commenced. The dishes were surrounded with black paper as explained above, and protected from dust. Twice weekly the liquids were renewed, and a weekly record was made of the number of fully-opened leaves in each dish. At the end of seven



weeks the plants in Series II had completely filled their dishes, so they were all halved as exactly as possible and the dry weight of one half of each dish estimated. This was repeated at the end of the eighth and eleventh weeks respectively. The experiment lasted for twelve weeks altogether, and the following figures were obtained :

TABLE III.

<i>No. of Leaves at end of</i>	<i>Series I. Detmer's Solution. Dish Number.</i>						<i>Series II. Detmer's Solution + Bacterized Peat. Dish Number.</i>					
	1	2	3	4	5	Mean.	6	7	8	9	10	Mean.
1st week	15	13	12	14	14	13.6	12	17	16	15	19	15.8
2nd "	21	20	17	20	18	19.2	21	25	24	21	27	23.6
3rd "	29	27	24	29	28	27.4	32	34	31	33	36	33.2
4th "	41	40	41	40	42	40.8	51	54	53	56	55	53.8
5th "	48	49	45	52	48	48.4	64	73	62	73	73	69.0
6th "	62	69	61	69	62	64.6	92	107	105	104	114	104.4
7th "	84	98	84	110	92	93.6	142	136	144	148	152	144.4
8th "	120	144	128	156	148	139.2	212	236	232	256	252	237.6
9th "	152	180	152	180	164	165.6	272	336	304	344	348	320.8
10th "	160	180	152	192	168	170.4	284	376	344	388	372	352.8
11th "	160	192	168	216	176	182.4	320	400	384	456	408	393.6
12th "	168	224	176	240	176	196.8	392	440	408	496	464	440.0

It will be observed that the rate of growth of the plants diminished in both series towards the end of the experiment, owing to the lowering of the temperature during October and November. The figures obtained for the dry weights of the whole of the plants in the two series, calculated from the weights of the halves on the three occasions on which the plants were divided, are as follows :

TABLE IV.

<i>Weight of Plants in mg. at end of</i>	<i>Series I. Dish Number.</i>						<i>Series II. Dish Number.</i>					
	1	2	3	4	5	Mean.	6	7	8	9	10	Mean.
7th week	80.0	83.2	88.8	92.6	84.4	86.0	169.2	148.4	156.8	154.4	158.0	157.4
8th "	104.0	105.6	95.6	132.0	117.2	110.9	276.0	213.6	232.8	249.6	243.2	243.0
11th "	140.8	147.2	150.4	161.6	142.4	148.5	465.6	556.8	526.4	574.4	549.2	532.5

The number of leaves in each dish at the beginning of the experiment, neglecting buds, was ten, and the average weight of the sets was 10.15 mg. This corresponds to an average weight for 100 leaves of 101.5 mg. at the beginning of the experiment. Comparing this with the calculated weight of 100 leaves at the end of the seventh, eighth, and eleventh weeks respectively, the following results are obtained :

TABLE V.

	<i>Series I.</i>			<i>Series II.</i>		
	<i>Average No. of Leaves.</i>	<i>Average Weight. mg.</i>	<i>Calculated Weight of 100 Leaves. mg.</i>	<i>Average No. of Leaves.</i>	<i>Average Weight. mg.</i>	<i>Calculated Weight of 100 Leaves. mg.</i>
At beginning	10	10.15	101.5	10	10.15	101.5
7th week	93.6	86.9	91.8	144.4	157.4	109.0
8th "	139.2	110.9	79.7	237.6	243.0	102.3
11th "	182.4	148.5	81.4	393.6	532.5	135.3

All the above figures indicate that, as in *Lemna*, not only is the rate of multiplication less in Detmer's solution without the presence of the growth-promoting organic substances, but there is also a diminution in size of the individual leaves as indicated by their weight. These facts were also very evident from the general appearance of the plants. The original plants placed in mineral nutrients only remained fairly healthy throughout the experiment, but the new leaves arising from them became successively smaller and more yellow in colour, indicating some interference with the metabolic activities of the plants. Those supplied with the organic substances remained perfectly healthy throughout the experiment, the new leaves arising becoming fully as large as, and in some cases larger than, the original ones. Pl. XVII, Fig. 3, shows a photograph of the whole set of dishes, taken in the last week of the experiment, and Pl. XVII, Fig. 4, shows one representative dish from each series on a larger scale taken at the same time. Both the difference in rate of multiplication of the leaves and the variation in their size are clearly shown in these two photographs.

#### EXPERIMENT WITH *AZOLLA FILICULOIDES*.

A set of ten dishes, divided into two series of five dishes each, was prepared in precisely the same way as for the *Salvinia* experiment. Into each of these dishes were counted out ten small sprigs of *Azolla filiculoides*, the sprigs being as nearly alike as possible. Since it was impossible to estimate the number of leaves on this plant, the number of visible growing-points was counted, and the total number of growing-points introduced into each dish on the ten small portions of *Azolla* was eighty. Four similar sets were counted out at the same time, and their dry weight was estimated. The figures obtained were 16.4, 17.0, 16.8, and 16.6 mg. respectively, giving an average of 16.7 mg. as the dry weight of the original contents of each dish.

The culture solutions were changed twice weekly and the number of visible shoots counted once weekly. At the end of the fourth week the plants in Series II had filled their dishes, so the contents of all the dishes were halved, one half being retained and the dry weight of the other half estimated. This was repeated at the end of the fifth, sixth, eighth, and eleventh weeks.

The weekly figures for each dish are shown in the table below, the number of shoots being given in the upper portion of the table and the weights in the lower part. The figures given represent those for the complete set each week, and not for the fractions of the complete sets (one-thirty-second part) which were actually in the dishes after the fourth week.

The contents of dishes No. 3, 4, 8, and 10 were pressed and mounted as a permanent record, and photographs of these pressed plants are shown in Pl. XVII, Figs. 5 and 6.

TABLE VI.

No. of Shoots.	Series I. Detmer's Solution.										Series II. Detmer's Solution + Bacterized Peat.										Mean.
	Dish Number.										Dish Number.										
	1	2	3	4	5	Mean.	6	7	8	9	10		7	8	9	10		Mean.			
At beginning	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80	80		
1st week	213	253	239	239	239	237	217	240	239	216	256	234	240	239	216	256	234	240	239		
2nd "	360	371	366	406	346	370	350	388	440	362	440	396	388	440	362	440	396	388	440		
3rd "	475	437	505	524	501	488	552	666	642	573	632	613	666	642	573	632	613	666	642		
4th "	640	654	644	690	682	662	838	842	874	818	858	846	842	874	818	858	846	842	874		
5th "	896	860	772	832	868	846	1,264	1,212	1,172	1,132	1,392	1,234	1,212	1,172	1,132	1,392	1,234	1,212	1,172		
6th "	1,248	1,264	1,048	1,160	1,272	1,198	1,928	1,784	2,048	1,928	2,088	1,955	1,784	2,048	1,928	2,088	1,955	1,784	2,048		
7th "	2,032	1,784	1,784	1,840	1,960	1,880	2,704	2,696	3,096	2,992	3,696	3,037	2,696	3,096	2,992	3,696	3,037	2,696	3,096		
8th "	3,024	2,864	2,832	2,800	2,864	2,877	3,904	3,728	4,448	4,336	4,672	4,218	3,728	4,448	4,336	4,672	4,218	3,728	4,448		
9th "	4,160	4,016	3,664	3,712	3,856	3,882	5,488	8,312	5,536	5,968	6,480	5,757	8,312	5,536	5,968	6,480	5,757	8,312	5,536		
10th "	4,672	4,736	4,160	4,464	4,496	4,506	6,592	6,720	6,564	7,248	7,262	6,867	6,720	6,564	7,248	7,262	6,867	6,720	6,564		
11th "	5,600	5,440	5,536	5,408	5,504	5,498	10,272	10,656	9,632	10,112	10,112	10,157	10,656	9,632	10,112	10,112	10,157	10,656	9,632		
12th "	9,120	9,056	7,968	8,384	8,000	8,506	15,744	12,960	15,200	15,392	13,952	14,650	12,960	15,200	15,392	13,952	14,650	12,960	15,200		
Weight of Plants.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.		
At beginning	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7		
4th week	101.2	126.4	133.2	132.0	113.6	121.3	151.6	151.6	161.6	147.2	155.2	153.4	151.6	161.6	147.2	155.2	153.4	151.6	161.6		
5th "	193.6	195.2	160.8	172.0	199.2	184.2	272.8	265.6	265.6	235.2	288.0	265.4	265.6	265.6	235.2	288.0	265.4	265.6	265.6		
6th "	334.4	326.4	261.0	302.4	323.2	310.1	452.8	457.6	478.4	491.2	481.6	472.3	457.6	478.4	491.2	481.6	472.3	457.6	478.4		
8th "	748.8	672.0	716.8	774.4	726.4	727.7	867.2	800.0	1,072.0	902.4	1,043.2	937.0	867.2	1,072.0	902.4	1,043.2	937.0	867.2	1,072.0		
11th "	1,740.8	1,651.2	1,568.0	1,619.2	1,568.0	1,629.4	2,259.2	2,323.2	2,425.6	2,240.0	2,323.2	2,314.2	2,323.2	2,425.6	2,240.0	2,323.2	2,314.2	2,323.2	2,425.6		

A comparison between the number of shoots in the two series is readily obtained by putting the average number in Series I at 100 for each week, when the corresponding numbers for Series II are obtained for the twelve weeks—99, 107, 126, 128, 146, 163, 161, 147, 148, 152, 185, 172. The fluctuation in these ratios from week to week is no doubt due to the unequal growth which had taken place during the varying weather conditions.

A corresponding comparison between the weights of the plants, putting the weight in both series at 100 for the beginning of the experiment, shows for Series II for the 4th, 5th, 6th, 8th, and 11th weeks respectively, the figures 126, 144, 152, 129, and 142.

It is evident from these figures that the plants of *Azolla filiculoides* respond to the presence of organic growth-promoting substances, although the response is not so marked as in the case of *Salvinia* and *Lemna*. *Azolla* plants, however, have long been known to be associated with an alga, *Anabaena*, which inhabits special cavities in the plants; and these cavities have also been shown by the writer<sup>1</sup> to contain nitrogen-fixing organisms. The symbiotic nature of these plants is a factor which must be taken into consideration in any discussion of their nutrition, since it is quite probable that such an association may furnish the plant with a proportion of the necessary organic substances for its metabolism. Under these circumstances a smaller response to the addition of such substances than is the case in a normal plant would be expected; and it was the fact that these plants, in the mineral solution only, retained their healthy appearance to a much greater extent than in the case of the plants with which the previous trials were carried out.

The increase in weight of these *Azolla* plants grown in the solution containing organic matter was not quite commensurate with the increase in number of the shoots, but this is readily explained by the fact that the main shoots of the control series elongated rather more than did those in Series II, before giving rise to secondary shoots. Hence the average weight of the shoots in Series I is greater than that in Series II, although in the latter series the total weight and number of the shoots are markedly superior to those in Series I, indicating a better nutrition of the plants.

#### EXPERIMENT WITH *LIMNOBIUM STOLONIFERUM*.

On September 22 a number of small plants of *Limnobium stoloniferum* were obtained, sufficient to start two small series of four dishes each. Four of these dishes (Series I) contained 300 c.c. of Detmer's solution, and the other four (Series II) 300 c.c. of this solution containing also the extract of one gramme of bacterized peat in every 1,000 c.c. Into each dish six plants of *Limnobium* were put, care being taken to select all eight sets as

<sup>1</sup> Bottomley, W. B.: Report Brit. Ass., 1910, pp. 786-7.

equally as possible. Each dish contained three plants with two leaves each, one with three leaves, and two with four leaves, a total of six plants, bearing between them seventeen leaves, in each dish. The solutions were renewed twice weekly, and a weekly record was made of the number of plants and leaves. During the fourth week of the experiment the dishes were found to be practically full, so the contents of each dish were halved as exactly as possible on October 16. The dry weight of one half of each was estimated, and a calculation made of the total weight in the dish. The other half of each set was retained and the experiment continued until Nov. 4, when growth was found to have practically ceased, for the experiment was carried out in an unheated greenhouse. The plants in each dish were counted, and the dry weight of the whole was determined. The results obtained are given below, and represent the figures corresponding to the complete sets at the respective dates.

TABLE VII.

Series.	Dish No.	Sept. 22.		Oct. 16.		Weight of set. gm.	Nov. 4.		Weight of set. gm.
		Number of Plants.	Leaves.	Number of Plants.	Leaves.		Number of Plants.	Leaves.	
I.									
Detmer's solution	1	6	17	40	108	0.1612	74	184	0.3232
	2	6	17	38	100	0.1496	66	192	0.3172
	3	6	17	36	92	0.1752	68	180	0.3304
	4	6	17	36	100	0.1728	56	174	0.2968
	Mean	6	17	37.5	100	0.1647	66	182.5	0.3169
II.									
Detmer's solution + bacterized peat	5	6	17	46	110	0.1896	86	248	0.4840
	6	6	17	48	108	0.1672	76	222	0.4560
	7	6	17	42	102	0.2092	76	202	0.4388
	8	6	17	44	104	0.1780	78	202	0.4556
	Mean	6	17	45	106	0.1860	79	218.5	0.4586

Thus on October 16, after three weeks' growth, Series II, with organic matter, showed an increase of 12 per cent. in the dry weight of the plants, and 6 per cent. in the number of leaves; but on Nov. 4, after a further three weeks' growth, the increase had amounted to 44.7 per cent. on the dry weight and 19 per cent. on the number of leaves. The plants in Series II appeared to be much more healthy than those in Series I, for the latter became very yellowish in appearance after the second week. The addition of the organic matter had a most marked effect on the health of the plants.

## CONCLUSION.

The experiments recorded above demonstrate that the water plants employed all require a certain quantity of organic substance for their proper growth and development, and that they are unable to develop to their full extent in nutrient solutions containing minerals only. The maximum

quantity of organic substance added in the above experiments never exceeded 184 parts per million, while the total concentration of mineral nutrients reached 5,500 parts per million. The effect of the organic matter could therefore not be attributed to its nutrient value, and it evidently functions as a growth-promoting substance, enabling the plants to make full use of the mineral substances supplied.

These plants would normally obtain such organic substance in the water of the ponds, &c., in which they grow, and it is noteworthy that the slower the rate of multiplication of the particular plant employed, the longer is the period that elapses before it begins to appreciate the deficiency of the mineral nutrient solution, and to respond to the addition of organic substance. Such a plant as *Lemna minor* or *major*, which normally multiplies very rapidly, will exhaust its original supply of these necessary growth-promoting substances, and will therefore respond to their addition to the nutrient solution, much more quickly than will such a plant as *Limnobium*, which grows and multiplies at a comparatively slow rate.

The plants chosen in these experiments are such as would permit of a fairly accurate estimate of their growth by counting and weighing, but similar results have been obtained with other plants, such as *Veronica beccabunga*, and the plants used are sufficiently diverse in nature to warrant the conclusion that all water plants require organic substances for their proper growth and development. It also cannot be expected that water plants differ so fundamentally from all other plants as to stand alone in such a requirement, and the author has already shown<sup>1</sup> that plants such as wheat seedlings, when the supply of organic material stored in their endosperm has been removed, respond very readily to the addition of such substances to the nutrient solution. An extension of these experiments with land plants, and particularly with cuttings, which have no seed to store up a quantity of organic substance, has been in progress for the last two years and will be reported in due course. The results of these experiments indicate that the same principle—the necessity for organic substance in small quantity—applies to ordinary land plants as well as to the water plants for which it has been established in the experiments recorded above.

#### SUMMARY.

*Lemna major*, in common with *Lemna minor*, was found to be quite unable to maintain its normal health in water-culture solutions containing mineral nutrients only. Immature plants used at the beginning of the experiment failed to reach the adult size, while adult plants began to bud, but the young plants thus formed never matured.

<sup>1</sup> Bottomley, W. B.: Annals of Botany, vol. xxviii, No. cxi, pp. 531-40; Proc. Roy. Soc., B., vol. lxxxviii, pp. 237-47 (1914).

When organic substances were added to the culture solutions the plants, whether young or full-grown, grew quite normally and reproduced themselves rapidly, the offspring rapidly reaching the normal size.

The effective organic substances were found to be present in an auto-claved growth of *Azotobacter chroococcum*, crude nucleic acid derivatives from raw peat, and a water extract of bacterized peat.

Similar trials carried out with *Salvinia natans* showed that these plants also are unable to maintain themselves in health without the presence of organic growth-promoting substances. The plants originally placed in mineral nutrients only remained healthy, but all new leaves formed were successively smaller and more yellowish in appearance. The addition of organic matter to the solution resulted in a more rapid rate of growth, the new leaves formed rapidly reaching the full normal size, and remaining perfectly healthy in appearance.

*Azolla filiculoides* also responded to the presence of organic substances in the culture solution, although not to quite the same extent as in the case of the plants mentioned above. The control plants, in minerals only, remained healthy for a longer period than did the controls of these other plants, probably on account of the symbiotic habit of *Azolla*, which would of necessity result in the supply of a certain quantity of organic matter to the plants.

Plants of *Limnobium stoloniferum* were also experimented with, and it was found that the addition of organic substance materially improved the health and size, as indicated by weight, of the plants. In these, as in the preceding experiments, a marked effect of the organic addition was its influence on the colour of the plants, those in minerals only becoming yellowish after a time, while those supplied with organic substance retained their green colour.

In no case did the organic substance supplied exceed 184 parts per million, while the concentration of salts in the culture solution totalled 5,500 parts per million.

Throughout the experiment it was found that the more rapid the rate of multiplication of the plants, the quicker was the response to the addition of organic substance to the solution.

The plants, in nature, obtain their supplies from the water of the ponds and streams in which they grow, and the quantity of these organic substances present in their tissues at the beginning of the experiment will necessarily diminish more rapidly as their rate of multiplication is increased. The more rapidly-growing plants therefore respond more quickly to the addition of organic substances in their culture solutions.



## EXPLANATION OF PLATE XVII.

Illustrating Prof. Bottomley's paper on the Effect of Organic Matter on the Growth of various Water Plants in Culture Solution.

Fig. 1. *Lemna major*. Contents of dish No. 2 (Table II), after four weeks' growth. Product of 10 plants. About  $\frac{1}{2}$  natural size.

Fig. 2. *Lemna major*. Contents of dish No. 6 (Table II), after four weeks' growth. Product of 10 plants. About  $\frac{1}{2}$  natural size.

Fig. 3. *Salvinia natans*. Ten dishes (Table III), photographed after eleven weeks' growth. Upper five dishes contained mineral nutrients only, lower five contained same mineral nutrients + 184 parts per million of organic matter. Ten leaves originally placed in each dish. About  $\frac{1}{3}$  natural size.

Fig. 4. *Salvinia natans*. One dish from each of the two groups above, upper dish containing mineral nutrients only, lower one containing mineral nutrients + organic matter. About  $\frac{2}{3}$  natural size.

Fig. 5. *Azolla filiculoides*. Contents of dish No. 3 (Table VI), after twelve weeks' growth. One thirty-second part of product of 80 shoots. About  $\frac{2}{3}$  natural size.

Fig. 6. *Azolla filiculoides*. Contents of dish No. 10 (Table VI), after twelve weeks' growth. One thirty-second part of product of 80 shoots. About  $\frac{2}{3}$  natural size.



*Lemna major*.

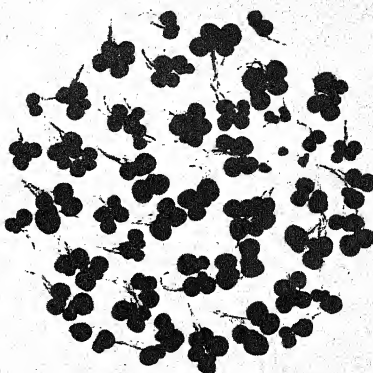
Set 2.



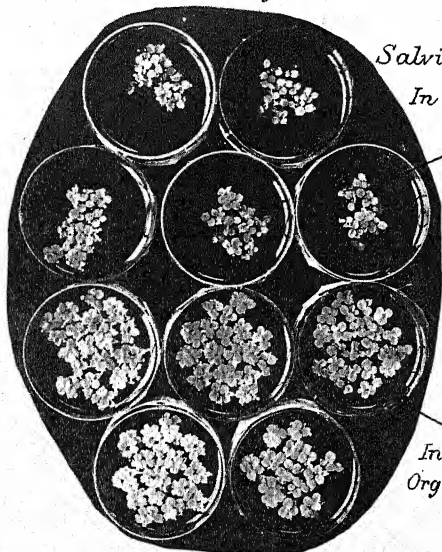
1.

*In Minerals only.**Lemna major*.

Set 6.



2.

*In Minerals + Organic Matter.**Salvinia natans*.*In Minerals only.*

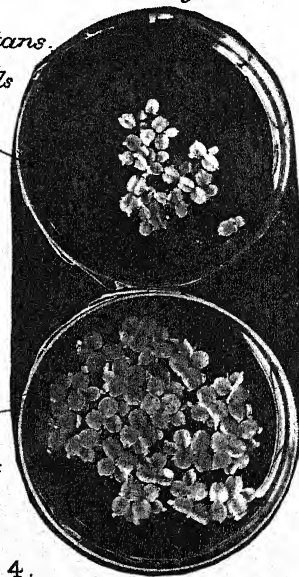
3.

*Azolla filiculoides*.

Dish 3.



5.

*In Minerals only*

4.

*Azolla filiculoides*.

Dish 10.



6.

*In Minerals + Organic Matter.*

Huth London.

Harold Welch Pe

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# Field Studies of the Carbon Dioxide Absorption of Coco-nut Leaves.

BY

F. T. McLEAN.

With Plate XVIII and nine Diagrams in the Text.

THE fixation of atmospheric carbon dioxide is undoubtedly the most important function of green plants. The rapidity of this process as it goes on in growing crops is an index of the food-manufacturing power of the plants. The effects of different environments and of different methods of culture on the carbon dioxide absorption may therefore serve as an immediate index of the value of such environment or treatment. This problem has been attacked in the past with a few crops by either analysing leaves gathered at intervals during the day<sup>1</sup> or by determining the increase in dry weight of leaves.<sup>2</sup>

Obviously neither of these methods can give a measure of the total photosynthesis of leaves attached to growing plants. Either the leaves must be detached, and then the accumulation of the products of photosynthesis interferes with the process unduly, as shown by Ewart;<sup>3</sup> or if the leaves remain on the plant, the translocation of materials away from them diminishes the accumulation and thus diminishes the apparent amount of photosynthesis as measured by either analysis or by dry weight increase. Apparently the most satisfactory method for measuring the process is by the determination of the gas exchange, or of one phase of it. Since the amount of carbon dioxide fixed by the plant is of more importance to plant nutrition than is the amount of oxygen evolved, it seems preferable to measure the carbon dioxide absorption. This can only be successfully accomplished by enclosing the leaf in some sort of chamber, thus altering conditions surrounding it and modifying the rate of carbon intake.

<sup>1</sup> A review of results obtained by this method is given in:

Atkins, W. R. G.: Some Recent Researches in Plant Physiology. Whittaker and Co., London, 1916.

<sup>2</sup> Thoday, D.: Experimental Researches on Vegetable Assimilation and Respiration. V. A Critical Examination of Sachs' Method for using Increase in Dry Weight as a Measure of Carbon Dioxide Assimilation in Leaves. Proc. Roy. Soc., London, B., lxxxii. 1-55, 1909.

<sup>3</sup> Ewart, A. J.: Assimilatory Inhibition in Plants. Jour. Linn. Soc. Bot., xxxi. 364-461, 1896.

The investigation here reported was undertaken to devise a method of measuring the carbon dioxide absorption of field crops *in situ* under conditions as little modified as possible. The study was carried out during class work in plant physiology at the College of Agriculture, Los Baños, Philippine Islands.<sup>1</sup>

#### METHOD.

The leaves to be studied were enclosed in a glass tube, 61 cm. long and 3.5 cm. in diameter (see Pl. XVIII, Fig. 1). The lower end of the tube enclosing the bases of the leaves was left open. The other end was fitted with a one-hole rubber stopper, through which was passed a 6 mm. glass tube connecting the large tube enclosing the leaf with the carbon dioxide extracting apparatus. Air was passed through this apparatus, which consisted of the following parts arranged in the order named: a series of three wash bottles, each containing 60 c.c. of sulphuric acid, a Pettenkofer tube containing 300 c.c. of barium hydroxide of known concentration (0.035 normal, more or less), a wash bottle containing an equal volume of the same solution, and a glass stop-cock to regulate the flow of air. The air then passed into an aspirator consisting of a large metal tank of about 280 litres capacity, so regulated by the glass stop-cock as to give a flow of nearly 19 litres of air per hour through the apparatus. A similar set of apparatus, connected to the same aspirator tank, but without a leaf in the open tube, was run beside it as a control.

The series of sulphuric acid bottles was introduced to equalize the moisture content of the air in the test and control apparatus, and also to prevent undue dilution of the barium hydroxide solutions in the Pettenkofer tubes. The air was introduced into the Pettenkofer tubes through glass tubes drawn out to 1 mm. top diameter. These regulated the size of the bubbles passing through the Pettenkofers so that they were about one centimetre long when they were flattened out along the tops of the tubes. Practically all of the carbon dioxide was removed from the air passing through the Pettenkofers. This was proved by the tests of the contents of the barium hydroxide wash bottles, which always showed a very small reduction in the alkalinity of the solution, and this reduction was found to be produced by the transfer of the solution from the bottle of stock solution to the wash bottle, and thence to the flask used for settling the solution after decanting. Furthermore the concentration of barium hydroxide in the wash bottles after each test was found to be identical for the test and the control apparatus, thus showing that the carbon dioxide content of the air leaving the apparatus, if there was any, was the same for test and control. The difference between the amounts of carbon dioxide absorbed

<sup>1</sup> The measurements here reported were made by Messrs. A. Mangoñón, T. Ventura, and G. Yap, working under my direction and supervision.

by the solutions in the Pettenkofer tubes at the end of any test period thus shows the difference in the carbon dioxide from a leaf and from the free air, i. e. the amount of carbon dioxide absorbed by the leaf from the air.

Diffusion of carbon dioxide from the outside air into the open end of the tube could not greatly increase the amount supplied to the leaf in this case, since the rate of flow of air into the tube was many times more rapid than the calculated rate of diffusion. Thus carbon dioxide was drawn in bodily into the apparatus at the rate of 6.2 m. per hour, many times as fast as it could possibly diffuse into the tube from a concentration of 0.0003 parts by volume to 0.00015 parts.

The conditions surrounding the leaves tested by this method may be summarized as follows: The air supplied to the outer end of the leaf had the same carbon dioxide content as the outside air. Since this air was moving through the tube at the rate of 6.2 metres per hour, it was constantly being renewed. The tip end of the leaf was supplied with air, the carbon dioxide content of which was never reduced by the activity of the leaf to as low as one-half of its original value in any of the tests of coco-nut or abaca leaves. Thus the carbon dioxide supply to the tested leaves was not reduced to very much less than normal. It was, however, seriously reduced for part of the time during some of the hours of the preliminary test of a sugar-cane leaf. The intensity of the light falling on the leaves was materially reduced by the glass tubes enclosing them. No measurements were made of the light intensity inside of the tubes. The effect of all of the modified conditions produced by the apparatus on the rate of carbon dioxide absorption may have been appreciable.

#### PROCEDURE WITH FIELD APPARATUS.

Each test was of either one or two hours' duration. Since the procedure was similar in all, and most of the tests were for two hours each, the latter only will be fully described. It was found during preliminary work in adjusting the apparatus that the rates of flow of air through the control and test apparatus could not be maintained exactly alike. The differences were due almost entirely to differences in the setting of the glass stop-cocks, which could not be adjusted precisely enough, and to the bubble tubes, which were not exactly alike. These also became somewhat coated with barium carbonate. This was removed at the end of each test by means of hydrochloric acid, and the tubes were rinsed before replacing them.

It is, of course, essential to the success of this method that the amount of air passing through the test and the control apparatus be measurably the same, since it is desired to compare the carbon dioxide content of equal volumes of air from the same general source, the one having subsequently been acted upon by a leaf, while the other was not thus modified. In order



to have the same volume of air pass through the two sets of apparatus during each test period, the following procedure was adopted.

At the end of the first half-hour the bubble tubes in the Pettenkofer tubes of the control and the test apparatus were interchanged, this operation necessitating the stopping of the flow of air for less than one minute. At the end of the first hour, the glass stop-cocks connecting the test and control apparatus with the aspirator were interchanged so that the valve which had regulated the flow through the test apparatus during the first hour would now regulate the flow through the control, and vice versa. At the same time that this was being done, the suction tube drawing air from both sets of apparatus was transferred to a full aspirator tank, so that the suction on the apparatus was the same at the beginning of the second hour as at the beginning of the first. These operations necessitated the stopping of the flow of air for nearly three minutes. At the end of the third half-hour the bubble tubes of the Pettenkofer tubes were again interchanged, thus being returned to their original positions as at the beginning of the test. By the above procedure the amount of air flowing through the two sets of apparatus was made measurably the same.

During each hour the rate of flow of air gradually diminished from twenty to eighteen litres per hour, due to loss of head in the aspirator tank. This hourly cycle in the rate of flow was of minor importance, since the rate was at all times sufficiently rapid to amply supply with air the leaves under test. This change in rate was moreover the same for both test and control.

At the end of each two-hour period, the Pettenkofer tubes and barium hydroxide wash bottles were renewed. The glass stop-cocks were returned to their original positions, the suction tube was attached to a full aspirator tank, and the bubble tubes used in the Pettenkofer tubes were cleaned, as stated above, before a new test was begun. All of these manipulations took, on an average, about five minutes.

#### TITRATION APPARATUS.

After removal from the apparatus the test and control Pettenkofer tubes and the barium hydroxide wash bottles were decanted into flasks which were closed and allowed to stand until the precipitated barium carbonate had settled out. Then the content of each flask was tested. Samples for testing were siphoned into a calibrated pipette, care being taken not to disturb the precipitated carbonate. The arrangement of this apparatus was similar to that employed by Copeland<sup>1</sup> to determine the concentration of barium hydroxide solution. It was so arranged that all of the air inside the apparatus was freed from carbon dioxide. The solution to be tested was first siphoned into a calibrated pipette, the capacity

<sup>1</sup> Copeland, E. B.: Chemical Stimulation and the Evolution of Carbon Dioxide. *Bot. Gaz.*, xxxv. 81-98, 160-83, 1903.

of which between two marks was 25.07 c.c. at 27° C. ; this pipette was filled with the solution and emptied three times to wash it before samples were drawn for testing. Then three samples of 25.07 c.c. each were drawn into Erlenmeyer flasks, two drops of neutral methyl orange were added to each, and they were promptly titrated against a standard solution of hydrochloric acid of nearly the same concentration as the barium hydroxide. The triplicate titrations agreed with each other within 0.05 c.c. of acid used in all except two cases, in which the divergent samples were 0.10 different from the two identical tests. The two identical numbers were assumed to represent the correct value.

#### EXPERIMENTATION.

After a large number of practice trials, tests were begun in May 1918, at the beginning of the south-east monsoon, which is also the beginning of the rainy season. Immediately preceding this time there was a drought extending from December 22, 1917, to May 13, 1918. Thus the vegetation was not in active growth at the beginning of these tests.

Nine series of tests are here reported, each consisting of observations during one daylight period. The first of these, Series 1, was a preliminary trial with detached sugar-cane leaves. Series 2 to 6 were made on coco-nut pinnae attached to the tree. Series 7 was on detached coco-nut pinnae, and Series 8 with an attached, and Series 9 with a detached abaca leaf (*Musa textilis*, Née).

All the measurements of attached coco-nut leaves were made<sup>1</sup> on one tree, about three years old, growing in an open closely-clipped lawn on shallow alluvial soil, in a flat plain at 55 m. elevation above sea-level. It was fully exposed on all sides. The tree was about seven feet high to the top of the tallest of its six leaves, and received no special attention, either by cultivation or irrigation, during the experiments.

#### SERIES 1.—*Tests of a half leaf detached from a sugar-cane plant,* *May 16, 1918.*

This experiment was undertaken to learn whether this gas analysis method gives approximately the same value for carbon dioxide absorption as is indicated by the increase in dry weight per unit area of the leaf, and also to get an idea of the effect of the conditions imposed by the apparatus on the increase in dry weight per unit area. The dry weight method of determining photosynthesis in sugar-cane was found to be inaccurate in recent work by Kuijper,<sup>1</sup> who states that in some cases the errors due to differences in weight of different parts of the leaf may attain to twenty-five

<sup>1</sup> Kuijper, J.: Proeven over de afhankelijkheid van het assimilatieproces bij het suikerriet van de uitwendige omstandigheden. Meded. van het proefstat. voor de Java-suikerind., Landb., ser. 1917, No. 13.

per cent. of the photosynthesis gain. His work makes the dry weight observations of doubtful value for precise comparisons, but as here used they have some interest for comparison with results by gas analysis. For this purpose two full-grown, dark green leaves were selected from healthy canes of about the same size, and of the same variety and age, growing side by side. These were removed from the plants at 5 a.m. on May 16. One side of each leaf was stripped from the midrib, their green weights and areas were determined, and then they were dried in a double-walled oven at 100°C. The remaining half-leaves, attached to the midribs, were placed with their cut ends in rain-water in glass phials, and one half-leaf was introduced into the test cylinder of the apparatus for determining the carbon dioxide absorption. The other was exposed beside the first, but left uncovered.

TABLE I.

Titration results from tests of carbon dioxide absorption by a detached half-leaf of sugar-cane on May 14, 1918. Area of half-leaf, 194.9 sq. cm. (25.07 c.c. Ba(OH)<sub>2</sub> used in the apparatus was equivalent in concentration to 20.10 c.c. of 0.0432 normal HCl).

Hour of removal from apparatus.	Amounts of acid in c.c. required to neutralize 25.07 c.c. of Ba(OH) <sub>2</sub> from Pettenkofer tubes of		Wash bottles of		Concentration of HCl used in titration.	Weather conditions.
	Test.	Control.	Test.	Control.		
A.M.						
7.30	17.60	17.55	19.85	19.85	0.0432	5.25 a.m., quite cloudy.
	17.65	17.55	19.85	19.90	"	6.45-7.45, bright sun.
	17.60	17.55	19.85	19.85	"	
9.30	19.70	18.00	19.90	19.85	"	7.45-8.20, dull.
	19.70	18.00	19.90	19.90	"	8.20-9.20, bright sun.
	19.70	18.00	19.90	19.90	"	
11.30	19.75	18.10	19.90	19.90	"	9.20-11, dull.
	19.70	18.05	19.90	19.90	"	11-11.35, bright sun.
	19.75	18.10	19.90	19.90	"	
P.M.						
1.30	19.50	18.10	19.95	19.90	"	11.35-11.48, dull.
	19.50	18.10	19.95	19.95	"	
	19.50	18.15	19.95	19.95	"	11.48-2.45, bright sun.
3.30	19.70	18.20	19.95	19.95	"	
	19.70	18.15	19.95	19.95	"	2.45-3, cloudy.
	19.75	18.20	19.95	19.95	"	3-3.35, bright sun.
5.30	21.05	19.35	21.25	21.25	0.0405	3.35-4.45, dull.
	21.05	19.35	21.25	21.20	"	
	21.05	19.40	21.25	21.25	"	4.45-6, bright sun one-half of the time.
6.30	20.30	20.25	21.25	21.25	"	
	20.30	20.25	21.30	21.25	"	
	20.30	20.25	21.25	21.30	"	6.30, light quite dim.

The apparatus was started at 5.30 a.m., and determinations of carbon dioxide absorption were made at two-hour intervals until 5.30 p.m. and again at 6.30 p.m. The results of the titrations of the samples obtained during these tests are given in Table I. Columns 2 and 3 show the amounts of acid required to neutralize the samples of barium hydroxide from the Pettenkofer tubes. Columns 4 and 5 show similar data for the corresponding barium hydroxide wash bottles. Column 6 shows the concentration of the hydrochloric acid used in titration, and column 7 gives observations and notes made during the tests.

The above data prove, first, that the difference in carbon dioxide content of the air leaving the test and control apparatus was inappreciable, since the titrations of the wash bottle solutions from test and control gave identical values for each period. This same uniformity in results from the wash bottles was obtained in all subsequent tests. The difference in titration values of solutions from the Pettenkofer tubes of test and control therefore indicates the difference in carbon dioxide content of the air passing through these, and thus serves as an index of the carbon dioxide absorbed by the leaf in the test apparatus.

The carbon dioxide absorption in grammes during each period is given in Table II (column 4). These values are all computed from the data of Table I.

To test whether the carbon dioxide of the air is completely extracted by the barium hydroxide in the Pettenkofer tubes, the difference in concentration between the Pettenkofer tube and wash bottle of the control for each period is computed to show the number of grammes of carbon dioxide absorbed per hour per c.m. of air (assuming that exactly 38 litres of air pass through the apparatus each two hours) (Table II, col. 2). This weight of carbon dioxide is then converted into terms of the number of parts by volume in 10,000 parts of the air, assuming a constant air temperature of 27° C., which is very nearly an average day temperature in this locality. These values are given in Table II, col. 3, and are sufficiently close to the assumed average carbon dioxide content of the air (3 parts in 10,000) to indicate that the carbon dioxide of the air was completely extracted. The calculated carbon dioxide content of the air was not found to be constant, but varied from 3 to 4 parts in 10,000. This variation is not surprising in view of the findings of Brown and Escombe, which show that the carbon dioxide content of the air near the earth varies as much as this. A part of this apparent variation in carbon dioxide content of the air may also have been due to variations in the rate of flow of the air through the apparatus from one period to another. Column 4 shows the difference in titration values between the control and the test apparatus. Column 5 shows the amount of carbon dioxide absorbed by the leaf per square metre.

TABLE II.

The amounts of carbon dioxide withdrawn from the air by the barium hydroxide and the amounts of carbon dioxide absorbed by the sugar-cane half-leaf, each two hours during May 14, 1918. (Computed from data given in Table I.)

Period.	CO <sub>2</sub> absorbed from air per hour.	Computed CO <sub>2</sub> content of air at 27° C. parts per 10,000.	Diff. in concentration of Ba(OH) <sub>2</sub> from test and blank Pettenkofer in c.c. of acid.	CO <sub>2</sub> absorbed by leaf per sq. metre. grms.
5.30-7.30 a.m.	0.0262	3.90	0.05	0.029
7.30-9.30 "	0.0216	3.21	1.70	0.992
9.30-11.30 "	0.0205	3.04	1.65	0.963
11.30 a.m.-1.30 p.m.	0.0210	3.13	1.40	0.817
1.30-3.30 p.m.	0.0199	2.96	1.50	0.875
3.30-5.30 "	0.0203	3.02	1.70	0.930
5.30-6.30 "	0.0214	3.18	0.05	0.027

Total 13 hrs.

4.633

The march of the carbon dioxide absorption as shown by the apparatus, for a period of 13 hours of daylight, is presented graphically in Diagram 1, in

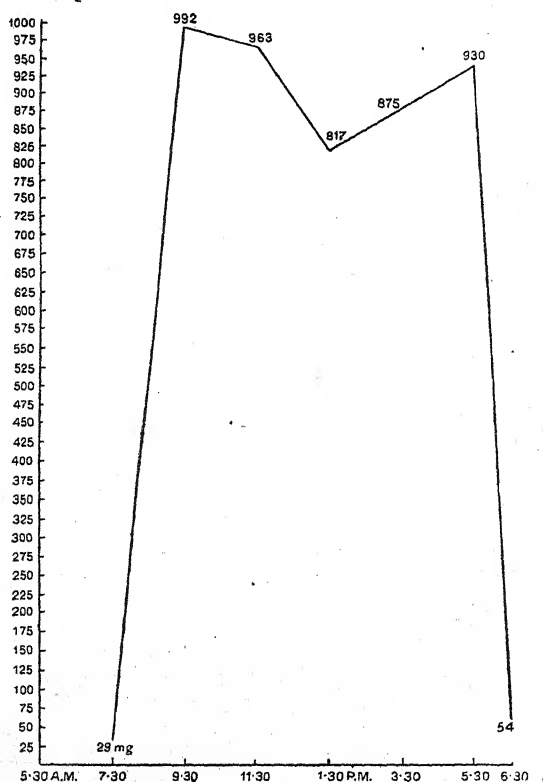


DIAGRAM 1. Amounts of carbon dioxide absorbed per sq. m. during two-hour periods by a detached sugar-cane leaf on May 14, 1918.

which the abscissae represent the hours at which each test period ended, and the ordinates represent rates of carbon dioxide absorption in grammes per square metre per two hours.

It will be seen that the amounts of carbon dioxide absorption from 5.30 to 7.30 a.m. and again from 5.30 to 6.30 p.m. were negligible, since they represented in both cases the minimum amounts of acid which were measured in the titrations, i.e. 0.05 c.c. Thus the really effective period of carbon dioxide absorption was during ten hours, from 7.30 a.m. to 5.30 p.m., and the maximum rates of absorption occurred at 7.30 to 9.30 a.m. and 3.30 to 5.30 p.m., there being thus a midday depression which reached its lowest point at 11.30 a.m. to 1.30 p.m. This depression was during an interval of bright sunshine, as shown by the observations in Table I, and was also at the time when the sun is most directly overhead, so it may have been caused by excessive insolation. Or it is quite as possible that something connected with the internal metabolism of the leaf, and not any particular set of atmospheric conditions, may be responsible for this rather peculiar behaviour.

For an approximate comparison of carbon dioxide absorption and the gain in dry weight of the leaf, the two leaves used in this experiment were plucked in the morning. One half of each was measured, weighed, and dried in the morning, and the remaining halves were exposed; one inside the apparatus (test leaf), and one outside (exposed leaf) beside it, as was stated above. These latter were measured and dried at 6.30 p.m. The results are given below.

TABLE III.

Comparison of increase in dry weight of detached half-leaves, one inside the glass tube of the apparatus and the other fully exposed to the sun during one day.

Leaf designation.	Dried at	Areas in sq. cm.	Dry wt. in grm.	Dry wt. per sq. m. in grm.	Apparent gain in dry wt. per sq. m. grm.
$\frac{1}{2}$ of tested leaf.	6.30 p.m.	194.87	1.416	72.66	
" " $\frac{1}{2}$ leaf	5.30 a.m.	171.63	1.172	68.22	4.44
$\frac{1}{2}$ of exposed leaf	6.30 p.m.	160.53	1.204	75.0	
" " $\frac{1}{2}$ leaf	5.30 a.m.	186.77	1.316	70.46	4.54

The apparent gain in dry weight of the leaf inside the apparatus was thus 4.44 grm. per sq. m. But Kuijper has shown<sup>1</sup> that usually the outer side of the sugar-cane leaf, which is the side covering the rolled leaf in the bud, is smaller in area, has more veins per unit area, and is usually heavier in dry weight per sq. m. than the inner side by about 1.6 grm., in at least one variety of cane. It has also been shown by Thoday<sup>2</sup> that most leaves shrink during the day, sometimes as much as 1 per cent. of their

<sup>1</sup> Kuijper, J., cited above on p. 371.

<sup>2</sup> Thoday, D., cited above on p. 367.

area. This also may have caused an error in this experiment, since the area of each half-leaf was measured just before drying it.

Since the outer side of the leaf was dried in the morning in the case of the test leaf, the gain in dry weight is undoubtedly too low on this account. On the other hand, the shrinkage and consequent gain in dry weight per unit area would tend to partially counterbalance this. However, with errors of the magnitude of those pointed out in the foregoing, no close agreement is to be anticipated between the gain in dry weight per unit area and the amount of carbon dioxide absorbed. A rough similarity in the magnitude of the calculated gains in weight per sq. m. by the dry weight method and by the gas analysis method is clearly shown.

The difference in weight of the outer and inner sides of a sugar-cane leaf also affects the comparison of the tested and the exposed leaf, for in the tested leaf the outer (heavier) half was dried in the morning, leaving the inner (lighter) half for the test. This would tend to make the observed increase in dry weight too small. In the case of the exposed leaf the reverse procedure was followed, the inner (lighter) side being dried in the morning, thus tending to make the result too high. For this reason the observed difference in increase in dry weight (0.10 gm.) between the exposed leaf and the tested one is not significant, being due possibly to the error as explained above.

SERIES 2 TO 6.—*Tests of coco-nut pinnae attached to the tree.*

Five of the six apparently healthy leaves of a young coco-nut tree were tested during these series. These leaves were numbered 1 to 6 from the youngest to the oldest, as shown in Plate XVIII, Fig. 2, which is a photograph of the tree, taken on May 28, at 5 p.m., during the sixth series of tests with leaf No. 4.

These tests were all performed in a similar manner to those described under series 1, except that the leaves used were attached to the plant. The width of spread of the edges of adjacent pinnae were measured, when possible, at each observation period, to compare the rate of photosynthesis to the changes in water content of the leaves. Each pinna has a cross-section like an inverted V, and this V becomes narrowed towards midday and again expands at night, due to expansion and contraction of the hinge cells at the angle on both sides of the midrib. This spread was measured at three marked points on each of two pinnae, and the changes in the average values computed for each test period are used as indices of the water content of the leaves.<sup>1</sup>

The observed data are presented in Table IV, and the computed carbon dioxide absorption values are summarized in Table V. Table IV is similar in data and arrangement to Table I, except that the concentration of acid

<sup>1</sup> Copeland, E. B.: *The Coconut*. Macmillan and Co., London, 1914, p. 11.



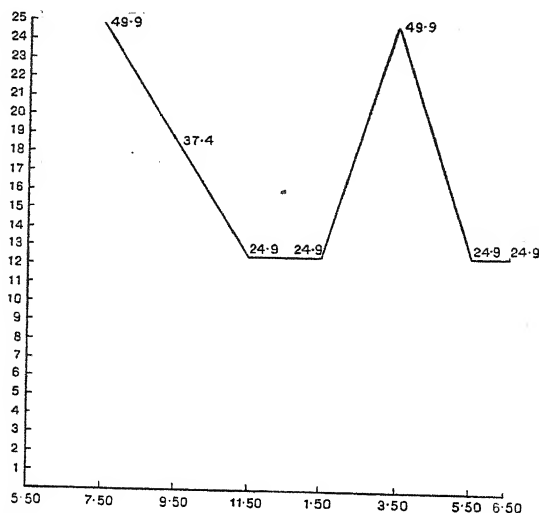


DIAGRAM 2. Amounts of carbon dioxide absorbed per square metre each two hours by two pinnae of coco-nut on May 23, 1918.

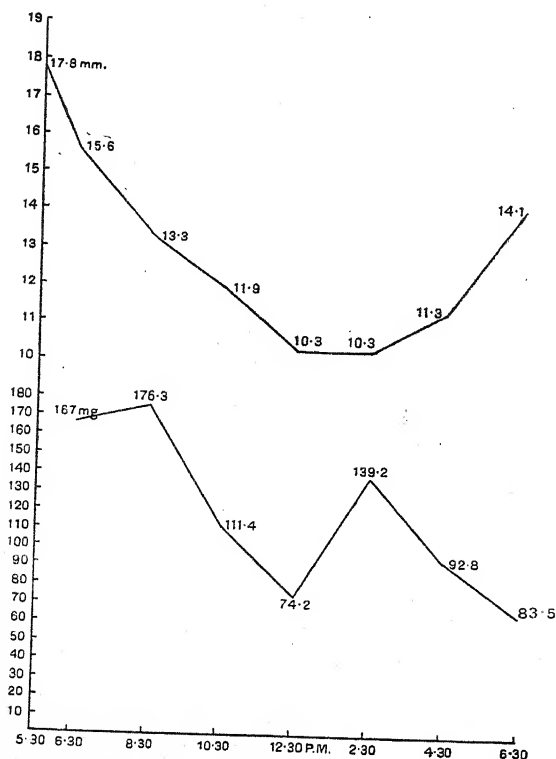


DIAGRAM 3. Amounts of carbon dioxide absorbed per square metre each two hours by two pinnae of coco-nut (lower graph) and spread of edges of pinnae (upper graph) on May 16, 1918.

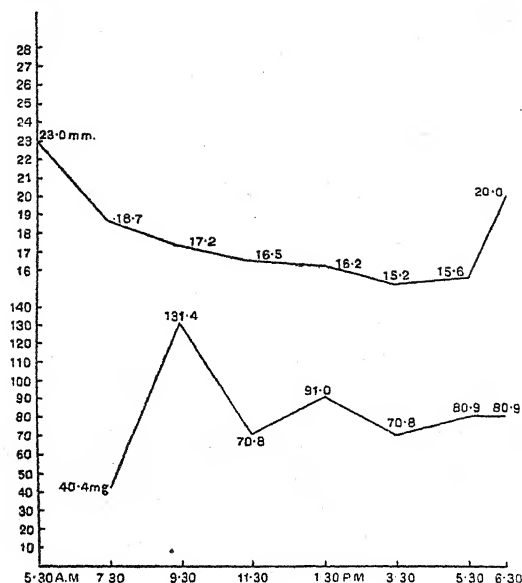


DIAGRAM 4. Amounts of carbon dioxide absorbed per square metre each two hours by two pinnae of coco-nut (lower graph) and spread of edges of pinnae (upper graph) on May 25, 1918.

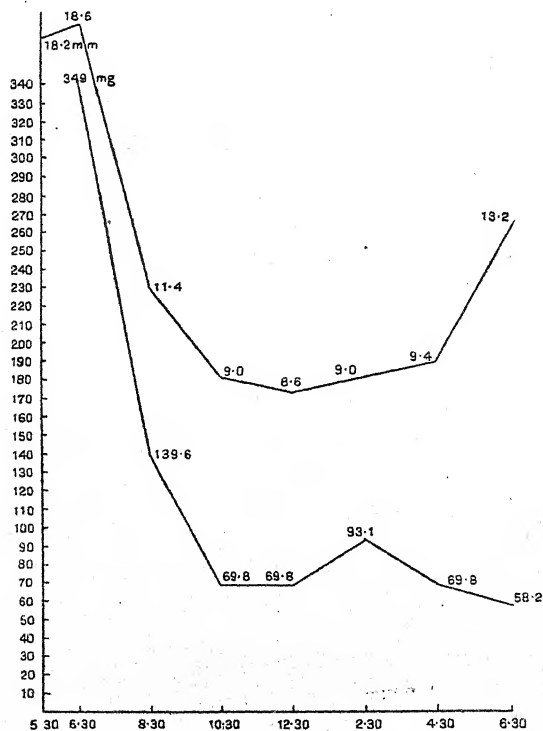


DIAGRAM 5. Amounts of carbon dioxide absorbed per square metre each two hours by two pinnae of coco-nut (lower graph) and spread of edges of pinnae (upper graph) on May 28, 1918.

is not stated, because 0.0405 normal hydrochloric acid was used throughout. In Table IV, column 1 gives the hour at which each observation period ended. Each period was of one or two hours' duration, and observations began at daylight, at 5.30 a.m., and continued until 6.30 p.m. in all except series 4, on May 24, which was begun at 5.50 a.m. and ended at 6.50 p.m. Columns 2 and 3 give the amounts of acid required to neutralize the barium hydroxide drawn from the Pettenkofer tubes of test apparatus and of control apparatus respectively. These are the final values for each test, and represent the most probable value of three titrations, at least two of which were identical in each case. Columns 4, 5, 6, and 7 give data for the spread of two pinnae adjacent to the ones tested and on the same compound leaf. Each pinna was measured repeatedly at three marked points, and the average of the six values thus obtained at each time of measurement is plotted as spread of pinnae on the graphs (Diagrams 2 to 6). Column 8, 'Weather conditions', shows the observed sunlight condition during each day. The conditions are classified as bright, dull, or cloudy. *Bright* indicates full sunshine. *Dull* indicates that light clouds made the shadows cast by the sun dim but not entirely obscured. *Cloudy* indicates that heavy clouds obscured the sun.

In Table V are shown the amount of carbon dioxide absorbed in grammes (col. 2) and the spread of the pinnae in centimetres (col. 3), both computed from Table IV. There are also included such data from weather instruments as were available for the days in question.

TABLE IV.

Titration results from tests of carbon dioxide absorption by leaves of a young coco-nut tree, May 16 to 28, 1918.

SERIES 2.—Two pinnae of leaf 2, 574.32 sq. cm. Tested on May 16.

Hour of removal from apparatus.	Acid required to neutralize 25.07 c.c. of Ba(OH) <sub>2</sub> .		Time measured.	Spread of lower edges of adjacent pinnae.			Weather conditions.
	Test.	Control.		cm.	cm.	cm.	
A.M.	c.c.	c.c.					
			5.40	1.70	2.0	2.30	
6.30	19.85	19.40	"	1.40	1.50	1.80	5.45, sun up.
			6.40	1.60	1.70	2.05	
8.30	20.35	19.40	"	1.30	1.30	1.60	6.10, sunlight on leaf.
			8.35	1.35	1.50	1.70	Bright sunlight all day.
10.30	20.10	19.50	"	1.10	1.10	1.25	
			10.30	1.20	1.30	1.60	
P.M.			"	1.10	0.90	1.05	
12.30	19.90	19.50	12.30	1.0	1.05	1.45	
			"	0.90	0.80	1.0	
2.30	20.15	19.40	2.30	0.90	1.0	1.50	
			"	0.90	0.90	1.0	
4.30	20.05	19.55	4.30	1.10	1.20	1.55	
			"	1.10	1.0	0.85	
6.30	20.10	19.65	6.30	1.30	1.60	1.90	6.25, sun down.
			"	1.15	1.10	1.40	

TABLE IV (continued).

SERIES 3.—Three pinnae of leaf 6, 373.36 sq. cm. area.  
Tested on May 21.

Hour of removal from apparatus.	Acid required to neutralize 25.07 c.c. of $\text{Ba}(\text{OH})_2$ .		Time measured.	Spread of lower edges of adjacent pinnae.			Weather conditions.
	Test.	Control.		cm.	cm.	cm.	
A.M.	c.c.	c.c.					
6.30	16.50	16.70	6.30	2.70	2.90	2.85	5.45, sun up.
			"	2.85	3.0	3.10	6.15, sunlight on leaf.
8.30	16.80	16.70	8.30	2.30	2.50	2.50	8.20-8.40, dull.
			"	2.60	2.65	2.80	8.40-9.12, bright sun.
10.30	16.75	16.70	10.30	2.30	2.35	2.30	9.12-9.20, dull.
			"	2.50	2.45	2.50	9.20-11.04, bright sun.
P.M.							
12.30	16.85	16.65	12.30	2.10	2.20	2.25	11.04-11.10, dull.
			"	2.45	2.30	2.35	11.10-12, bright sun.
							12-12.05, dull.
2.30	16.90	16.85	2.30	1.90	2.10	2.25	12.05-2.20, bright sun.
			"	2.45	2.30	2.35	2.20-2.25, dull.
4.30	16.90	16.85	4.30	1.90	1.75	1.70	2.25-6.10, bright sun.
			"	2.35	2.30	2.35	
6.30	16.95	16.90	6.30	2.05	2.45	2.50	
			"	2.50	2.50	2.60	

SERIES 4.—Two pinnae of leaf No. 1, 428.05 sq. cm. area.  
Tested on May 23.

Hour of removal from apparatus.	Acid required to neutralize 25.07 c.c. of $\text{Ba}(\text{OH})_2$ .		Time measured.	Spread of lower edges of adjacent pinnae.	Weather conditions.
	Test.	Control.			
A.M.	c.c.	c.c.			
7.50	16.35	16.15	The pinnae had not yet spread.		5.50, sun out bright.
9.50	17.10	16.95			
11.50	17.20	17.10			
P.M.					
1.50	17.0	16.90			3.25, cloudy and windy. 4.0-5.17, bright sun. 5.17-5.55, dull. 5.55-6.09, bright sun. 6.09, sun down.
3.50	21.70	21.50			
5.50	21.70	21.60			
6.50	22.45	22.40			

TABLE IV (continued).

SERIES 5.—Two pinnae of leaf No. 3, 527.24 sq. cm. area.  
Tested May 25.

Hour of removal from apparatus.	Acid required to neutralize 25.07 c.c. of Ba(OH) <sub>2</sub> .		Time measured.	Spread of lower edges of adjacent pinnae.			Weather conditions.
	Test.	Control.		cm.	cm.	cm.	
A.M.	c.c.	c.c.					
			5.20	2.80	2.35	2.30	
7.30	21.40	21.20	"	2.30	2.0	1.95	5.38, sun up. Bright.
			7.30	2.60	1.90	1.60	
9.30	22.25	21.60	"	2.10	1.60	1.40	
			9.30	2.55	1.65	1.35	
11.30	22.0	21.65	"	2.20	1.50	1.10	
			11.30	2.50	1.60	1.20	
P.M.			"	2.15	1.35	1.10	
1.30	22.10	21.65					
			1.30	2.50	1.55	1.20	
3.30	22.25	21.90	"	2.15	1.25	1.10	
			3.30	2.40	1.45	1.10	
5.30	22.20	21.80	"	2.15	1.15	0.85	
			5.30	2.45	1.45	1.10	
6.30	22.45	22.25	"	2.10	1.30	0.95	
			6.30	2.60	2.15	1.70	6.15, sun down.
			"	2.30	2.80	1.45	

SERIES 6.—Two pinnae of leaf No. 4, 458.07 sq. cm. area.  
Tested May 28.

Hour of removal from apparatus.	Acid required to neutralize 25.07 c.c. of Ba(OH) <sub>2</sub> .		Time measured.	Spread of lower edges of adjacent pinnae.			Weather conditions.
	Test.	Control.		cm.	cm.	cm.	
A.M.	c.c.	c.c.					
			5.30	1.80	1.90	1.90	
6.30	21.55	20.80	"	1.75	1.80	1.80	5.30, sun up.
			6.30	2.0	1.90	1.85	
8.30	18.05	17.45	"	1.80	1.80	1.80	5.57, sunlight on leaf.
			8.30	1.35	1.20	0.90	
10.30	17.90	17.60	"	1.0	1.20	0.85	5.57-9.50, bright sun.
			10.30	1.10	1.10	0.70	
P.M.			"	0.80	1.0	0.75	9.50-10.30, dull.
12.30	17.90	17.60					
			12.30	1.10	1.0	0.70	
2.30	18.05	17.65	"	0.95	0.90	0.60	10.30-12.35, bright.
			2.30	1.10	1.0	0.65	
4.30	18.0	17.70	"	1.05	1.05	0.65	12.35-1.10, dull, cloudy, and showers.
			4.30	1.20	1.10	0.65	
6.30	17.90	17.65	"	1.10	0.95	0.65	1.15-4.35, bright.
			6.30	1.40	1.20	1.20	
			"	1.20	1.15	0.95	4.35-4.45, dull.
							6.14, sun down.

TABLE V.  
CO<sub>2</sub> Absorption and Spread of Pinnæ of Coco-nut Leaves.

Date.	Leaf 1. May 23.			Leaf 2. May 16.			Leaf 3. May 25.			Leaf 4. May 28.			Leaf 6. May 21.		
	Hour.	CO <sub>2</sub> abs. per sq. m. in gm.	Spread of pinnæ. mm.	Hour.	CO <sub>2</sub> abs. per sq. m. in gm.	Spread of pinnæ. mm.	Hour.	CO <sub>2</sub> abs. per sq. m. in gm.	Spread of pinnæ. mm.	Hour.	CO <sub>2</sub> abs. per sq. m. in gm.	Spread of pinnæ. mm.	Hour.	CO <sub>2</sub> abs. per sq. m. in gm.	Spread of pinnæ. mm.
A.M.	5.50	0.0499	0	5.30 to 6.30	0.0835	Time. mm. { 5.30 17.8 6.30 15.6 7.30 }	5.30 to 6.30	0.0404	{ 6.30 23.0 7.30 18.7 6.30 }	5.30 to 6.30	0.1745	18.6	—	—	-0.0571 <sup>1</sup>
	10														
	7.50														
9.50	0.0374	0	0	8.30	0.1763	8.30 13.3	9.30	0.1314	9.30 17.2	8.30	0.1396	11.4	—	—	25.6
11.50	0.0249	0	0	10.30	0.1114	10.30 11.9	11.30	0.0708	11.30 16.5	10.30	0.0698	9.0	—	—	24.0
P.M.															
1.50	0.0249	0	0	12.30	0.0742	12.30 10.3	1.30	0.0910	1.30 16.2	12.30	0.0698	8.6	—	—	22.7
3.50	0.0499	0	0	2.30	0.1392	10.3	3.30	0.0708	3.30 15.2	2.30	0.0931	9.0	—	—	22.2
5.50	0.0249	0	0	4.30	0.0928	11.3	5.30	0.0809	5.30 15.6	4.30	0.0698	9.4	—	—	20.6
6.50	0.0125	0	0	6.30	0.0835	14.1	6.30	0.0404	6.30 20.0	6.30	0.0582	13.2	—	—	24.3
Total 13 hrs. Possible errors of single readings	0.2244			0.7609				0.5257			0.6748				0.1439
	± 0.0093			± 0.0101			± 0.0116			± 0.0143					

<sup>1</sup> This negative value represents respiration, and therefore is excluded from the total amount of photosynthesis by leaf 6.

In Table V the total amounts of carbon dioxide absorbed per square metre of leaf surface are shown to be all very low ; the amount for the most rapid leaf, i.e. for leaf 2, is only about one-sixth of the corresponding value for the detached sugar-cane leaf. This indicates that either the rate of photosynthesis in coco-nut is very slow or the carbon supply is supplemented from some other source than from carbon dioxide absorbed from the air by the leaf. It is possible that the coco-nut, with its massive trunk, may store organic acids and reconvert them to carbohydrates during sunlight.

A comparison of the different leaves (though treated on different, but very similar days, as shown by the weather records and observations) indicates that the rate of photosynthesis is most rapid in the middle-aged leaves. Leaves 2, 3, and 4 agree in having a much more rapid rate of carbon dioxide absorption than leaves 1 and 6. Leaf 1 was markedly immature, the pinnae not yet being spread apart at the time of testing. Since the lower surface, bearing the majority of the stomata, was not yet even exposed to the air, the leaf was quite naturally sluggish. Leaf No. 6, although apparently healthy, had assumed a slightly yellowish-green tint, quite distinct from the brilliant dark green of leaves 2, 3, and 4. Thus coco-nut agrees in this respect with most other plants studied hitherto, in that the maximum rate of carbon dioxide absorption is performed by the young, but fully developed leaves.

The diurnal march of carbon dioxide absorption is best seen in the graphs, Diagrams 2 to 6, which are constructed like Diagram 1. All of these graphs, though differing in details, show an early maximum rate, followed by a depression in rate of carbon dioxide absorption in the middle part of the day, again followed by a secondary rise in the rate just prior to the decline towards sunset. In leaves 1, 2, and 4 this daily march is quite uniform, the first maximum occurring in the period from 5.30 to 7.30 a.m. and the second maximum at 1.30 to 3.30 p.m. In the test of leaf 3 this is less apparent, there being the first maximum between 7.30 and 9.30 a.m. and a much less pronounced second maximum at 11.30 to 1.30 p.m. Leaf 6 has a well-marked maximum between 10.30 a.m. and 12.30 p.m. The above data, all except leaf 6, show an initial maximum rate of carbon dioxide absorption by coco-nut, followed later in the day by a secondary maximum. This behaviour closely parallels the behaviour of many plants in transpiration, as shown by Shreve,<sup>1</sup> and might suggest that this phenomenon likewise may be due to incipient drying. To test this approximately the measurements of the spread of the coco-nut pinnae are introduced in Diagrams 3 to 6. The graphs of spread of pinnae show minima, i.e. minima of water content of leaves, at hours varying from 12.30 to 4.30 p.m., and these minima show no

<sup>1</sup> Shreve, E. B. : *The Daily March of Transpiration in a Desert Perennial*. Carnegie Inst., Wash., Publ. No. 194 (1914).



relation to either the minima or maxima of carbon dioxide absorption. Thus the changes in water content of the leaves did not appear to exert a controlling influence upon the rate of photosynthesis.

Other factors of the environment which have been shown to influence the rate of photosynthesis of green leaves are: carbon dioxide supply from the air, angle of incidence of the sun's rays, intensity and composition of sunlight. The carbon dioxide supplied to the plants during each test period is shown by the difference in titration numbers of the control Pettenkofer tube and the control wash bottle. These values are shown below in Table VI.

TABLE VI.

Changes in carbon dioxide supply during the tests of coco-nut leaves, as indicated by the difference in titration numbers of control Pettenkofer tubes and control wash bottles.

Leaf No. 1.		Leaf No. 2.		Leaf No. 3.		Leaf No. 4.		Leaf No. 6.	
Time period.	Titration diff. c.c. of acid.	Time period.	Titration diff. c.c. of acid.	Time period.	Titration diff. c.c. of acid.	Time period.	Titration diff. c.c. of acid.	Time period.	Titration diff. c.c. of acid.
5.50-7.50	2.2	5.30-6.30	1.8	5.30-7.30	1.7	5.30-6.30	1.6	5.30-6.30	2.0
7.50-9.50	1.4	6.30-8.30	1.85	7.30-9.30	1.4	8.30	1.6	8.30	1.25
9.50-11.50	1.3	8.30-10.30	1.80	9.30-11.30	1.35	10.30	1.6	10.30	1.15
11.50-1.50	1.5	10.30-12.30	1.80	11.30-1.30	1.35	12.30	1.7	12.30	1.15
1.50-3.50	1.45	12.30-2.30	1.85	1.30-3.30	1.05	2.30	1.45	2.30	1.10
3.50-5.50	1.3	2.30-4.30	1.80	3.30-5.30	1.15	4.30	1.5	4.30	1.10
5.50-6.50	0.6	4.30-6.30	1.75	5.30-6.30	0.75	6.30	1.55	6.30	1.15

It will be noted that in all cases the carbon dioxide supply per hour is greater at the first hour than for the succeeding hours, and in most cases is nearly twice as great.

It is probable that the carbon dioxide content of the air is actually higher at night and in the early morning, due to respiration from the dense vegetation, and that this amount decreases rapidly when the plants begin to absorb carbon dioxide actively in the morning.

The high rates of carbon dioxide absorption by the coco-nut leaves in the early morning may then be partly due to the presence of more carbon dioxide in the air at that time than later.

That this is not necessarily the most important factor is indicated by leaf 2, which absorbed carbon dioxide at a slower rate at 5.30 to 6.30 a.m. than at 6.30 to 8.30 a.m., in spite of the fact that the amount of carbon dioxide supplied from the air at 5.30 to 6.30 a.m., as indicated in Table VI, was nearly as great for that one hour (1.8 c.c. index) as for the two succeeding hours (1.85 c.c. index).

The second maximum in the afternoon cannot be explained by changes in carbon dioxide content of the air, since it occurs at a time when the latter is markedly low. A more reasonable hypothesis to account for the depression in rate of carbon dioxide absorption and subsequent increase in the

LEAF NO. 6.

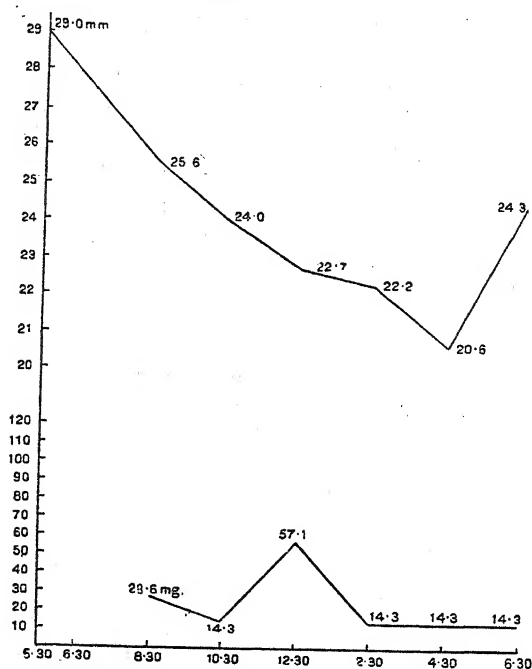


DIAGRAM 6. Amounts of carbon dioxide absorbed per square metre each two hours by three pinnae of coco-nut (lower graph) and spread of edges of pinnae (upper graph) on May 21, 1918.

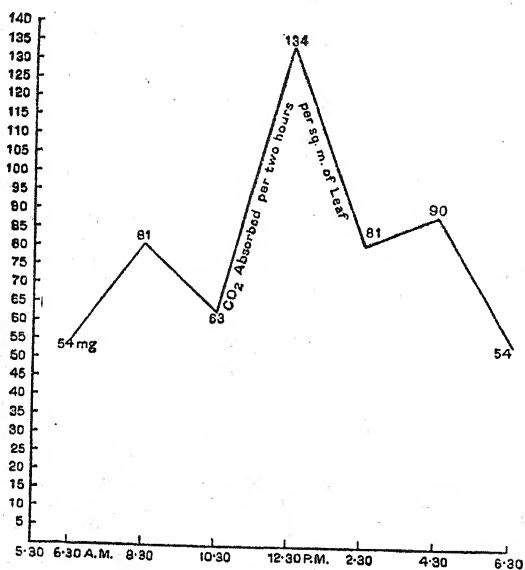


DIAGRAM 7. Amounts of carbon dioxide absorbed by two detached pinnae of coco nut on May 29, 1918.

afternoon is the decrease in the number of sun's rays striking each pinna at noon. The pinnae were placed with their long axes north and south in each test. The pinna is in cross-section like an inverted V. The incident rays strike one half vertically in the early morning, and the other half similarly in the afternoon. At noon, the sun's rays strike both sides obliquely, and also the actual area of the horizontal projection of each pinna is less than the area of one side of the pinna. Thus the low rate of carbon dioxide absorption at midday in these tests may be partly due to their position with respect to the sun's rays at that time. Since, however, the sugar-cane leaf, which lies flat and does not decrease its exposed surface at midday except by shrinkage, shows the same character of depression in rate of carbon dioxide absorption, it is necessary to assume, in this one case of sugar-cane at least, that the midday depression is due to a change in the internal condition of the leaf itself.<sup>1</sup>

#### TEST SERIES 7.

On May 29 two pinnae were detached from leaf 3 of a coco-nut tree growing near the one used in previous tests, and were placed in water. Then their rate of carbon dioxide absorption was tested for comparison with the attached leaves. The data obtained from these tests are presented in Table VII and Diagram 7, which are arranged similarly to the corresponding data in series 2 to 6 (Table V and Diagrams 2 to 6).

TABLE VII.

Absorption of carbon dioxide by two detached coco-nut pinnae of 595.6 sq. cm. area, during 13 hours on May 29, 1918.

Duration of Test.	Difference in concentration of $\text{Ba}(\text{OH})_2$ from test and blank in c.c. of 0.0405 normal $\text{HCl}$ .	$\text{CO}_2$ absorbed per sq. m.	$\text{CO}_2$ absorbed per sq. m. per hour.	Remarks.
		mg.	mg.	
5.30- 6.30 a.m.	0.15	27	27	Sun rose at 5.45.
6.30- 8.30 "	0.45	81	40	Struck leaf at 6.10
8.30-10.30 "	0.35	63	31	a.m. Bright sunshine
10.30 a.m.-12.30 p.m.	0.75	134	67	from 6.10 a.m. to
12.30-2.30 p.m.	0.45	81	40	1.30 p.m.
2.30-4.30 "	0.50	90	45	Light dull from 1.30
4.30-6.30 "	0.30	54	27	to 2.06. Rain 2.06-
				2.50 p.m.; dull 2.50-
		530 mg.		5.15 p.m. Bright
				sunshine from 5.15
				p.m. to 6.10 p.m.,
				when the sun set
				behind clouds.

The total amount of carbon dioxide absorbed per sq. m. by the detached pinnae, 530 mg., is thus almost the same as the amount absorbed

<sup>1</sup> For a report of a similar midday checking in photosynthesis of red clover, see Bot. Gaz., xxvi (1898), 347. The original text of this was not available at the time that the present paper was prepared.

during test series 5 by the pinnae of leaf No. 3, on May 25, i.e. 526 mg. The two leaves were of about the same age, each being third from the youngest. The character of the day was different in the two cases, however, for it was bright all day on May 25, but was dull during the afternoon on May 29. The test of the detached pinnae showed clearly that the total

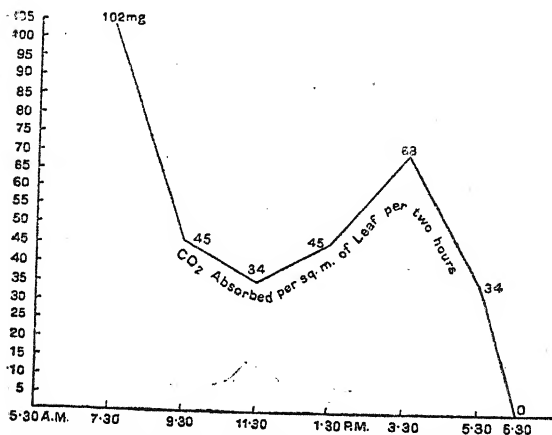


DIAGRAM 8. Amounts of carbon dioxide absorbed by a detached abaca leaf on May 18, 1918.

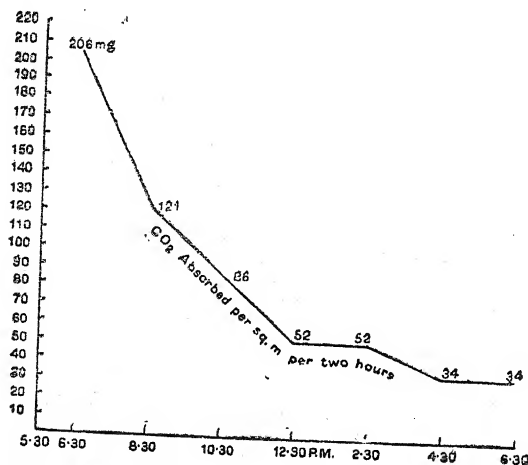


DIAGRAM 9. Amounts of carbon dioxide absorbed by an abaca leaf on May 31, 1918.

amount of assimilation was not seriously reduced by detaching the leaf. But the diurnal march of carbon dioxide absorption was very different in the detached pinnae from any of the pinnae attached to the plant, as is shown by Diagram 7. The graph of carbon dioxide absorption in this case is more irregular than those of Diagrams 2 to 6, and shows a very definite maximum at the 10.30 a.m. to 12.30 p.m. period, instead of two maxima with an intervening depression as was shown by most of the other leaves tested.

This exceptional behaviour of the detached pinnae indicates that possibly the midday decline in carbon dioxide absorption of the attached coco-nut leaves tested may have been due to incipient drying or some other unfavourable condition imposed upon the leaf by the condition of the rest of the plant.

#### SERIES 8 AND 9.

The two series of tests Nos. 8 and 9 of abaca leaves (*Musa textilis*) were made on a detached leaf on May 18 and on a leaf of a potted plant on May 31. In these tests the tubes used in the other tests to enclose the leaves were replaced by flat chambers 2 cm. deep and 90 cm. long with glass walls at top and bottom, the sides being supplied by movable wooden strips, fitted snugly against top and bottom plates by rubber strips. By this means the size of the chamber was adjusted to conform to the width of the leaves. Apart from this modification, necessitated by the broad abaca leaves, which resemble banana leaves, these tests were similar to those of sugar-cane and coco-nut. The results are shown below in Tables VIII and IX and Diagrams 8 and 9.

TABLE VIII.

Carbon dioxide absorption by a detached abaca leaf of 472.63 sq. cm. area on May 18, 1918.

Duration of Test.	Difference in concentration of $\text{Ba}(\text{OH})_2$ from test and control in c.c. of 0.0405 normal $\text{HCl}$ .	$\text{CO}_2$ absorbed per sq. m.	$\text{CO}_2$ absorbed per sq. m. per hour.	Remarks.
		mg.	mg.	
5.30-7.30 a.m.	0.45	102	51	Sunrise at 5.48.
7.30-9.30 "	0.20	45	23	Bright until 2.30 p.m.
9.30-11.30 "	0.15	34	17	
11.30 a.m.-1.30 p.m.	0.20	45	23	
1.30-3.30 p.m.	0.30	68	34	Rain 2.30 to 4 p.m.
3.30-5.30 "	0.15	34	17	Cloudy from 4 p.m. to the end of exp.
5.30-6.30 "	0	0	0	
328 mg.				

TABLE IX.

Carbon dioxide absorption by a leaf of 309.55 sq. cm. area of a potted abaca plant on May 31, 1918.

Duration of Test.	Difference in concentration of $\text{Ba}(\text{OH})_2$ from test and control in c.c. of 0.0405 normal $\text{HCl}$ .	$\text{CO}_2$ absorbed per sq. m.	$\text{CO}_2$ absorbed per sq. m. per hour.	Remarks.
		mg.	mg.	
5.30-6.30 a.m.	0.30	103	103	Sunrise at 5.45 a.m.
6.30-8.30 "	0.35	121	60	Sunshine on leaf at 6.10 a.m.
8.30-10.30 "	0.25	86	43	
10.30 a.m.-12.30 p.m.	0.15	52	26	Bright sunlight throughout the day.
12.30-2.30 p.m.	0.15	52	26	
2.30-4.30 "	0.10	34	17	
4.30-6.30 "	0.10	34	17	
482 mg.				

The above results show that the rates of absorption of abaca leaves are not widely different in full sunlight from those of coco-nut pinnae, and are much lower than that of sugar-cane. Further, each of the two abaca tests shows a maximum rate of carbon dioxide absorption very early in the morning, and the first, as shown in Diagram 8, has a second maximum at 1.30 to 3.30 p.m., which is not exhibited by the second abaca test (Diagram 9). Comparisons between these two tests would not be relevant, since the two leaves may not have been of comparable age, and no study was made of the effect of age on the photosynthetic activity of abaca leaves.

#### CONCLUSIONS.

1. The method here described for field studies of carbon dioxide absorption by leaves is satisfactory for comparative studies of the rates of carbon dioxide absorption by different leaves, by the same leaves at different times, and by the leaves of different kinds of plants.

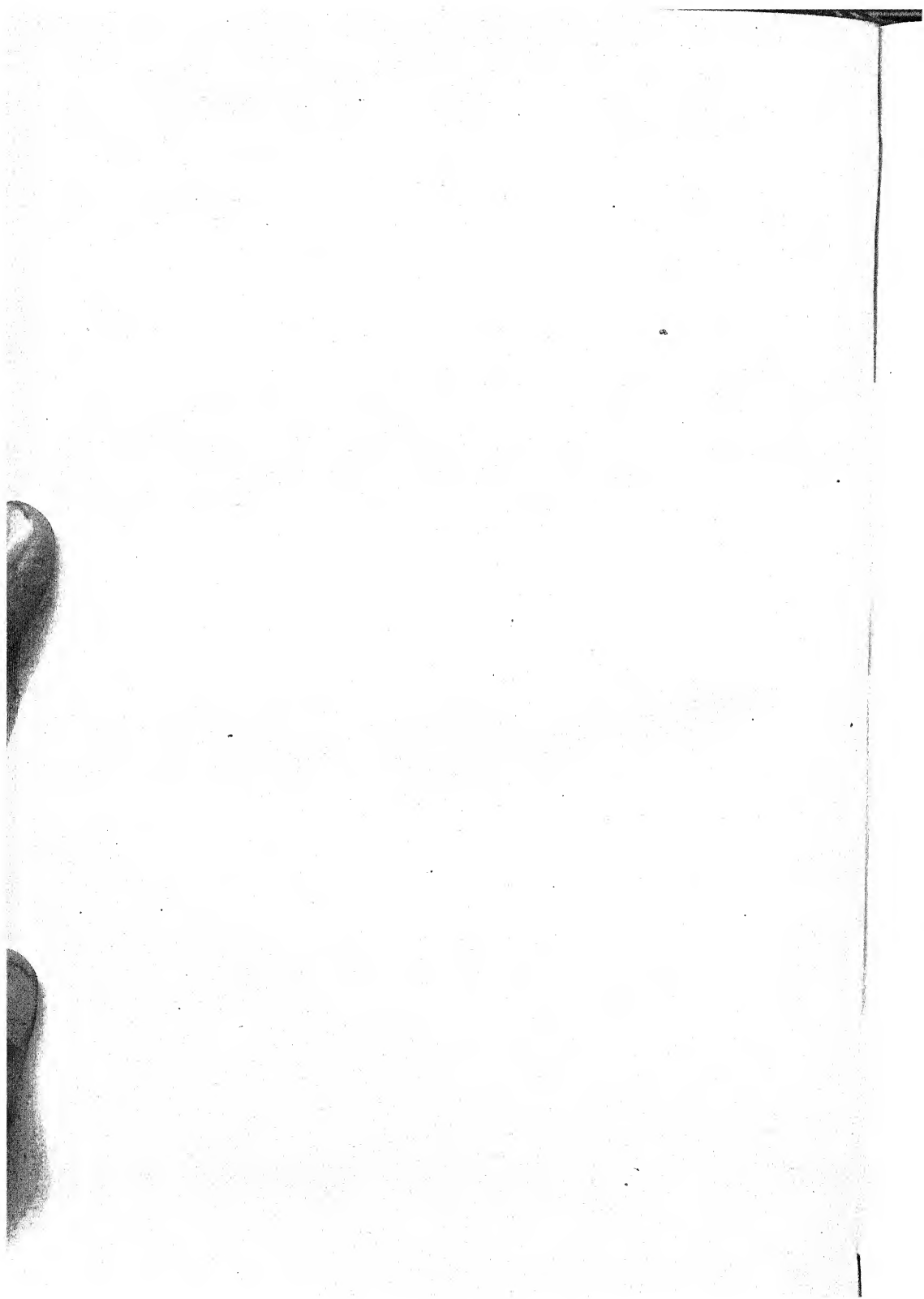
2. Middle-aged leaves absorbed carbon dioxide faster than either immature or old leaves. Of the five leaves of the coco-nut which were tested on one plant, the youngest leaf, which was not yet unfolded, and the oldest healthy-looking leaf (No. 6) both showed very low rates of absorption. The three young, fully expanded leaves all showed comparatively rapid rates.

3. The rates of absorption of carbon dioxide by coco-nut leaves show a maximum rate in the morning, a depression at midday, and a second rise in the afternoon, followed by a final decline towards sunset.

4. Detached pinnae of coco-nut absorb carbon dioxide at about the same rate as similar leaves attached to the plant, but the maximum occurs at a different time of day from that of attached coco-nut leaves.

5. Comparisons of carbon dioxide absorption by coco-nut leaves with that by the sugar-cane and abaca leaves tested indicate that sugar-cane absorbs much more rapidly than coco-nut under the conditions encountered during these tests.

UNIVERSITY OF PHILIPPINES,  
COLLEGE OF AGRICULTURE,  
LOS BAÑOS.  
March 20, 1918.





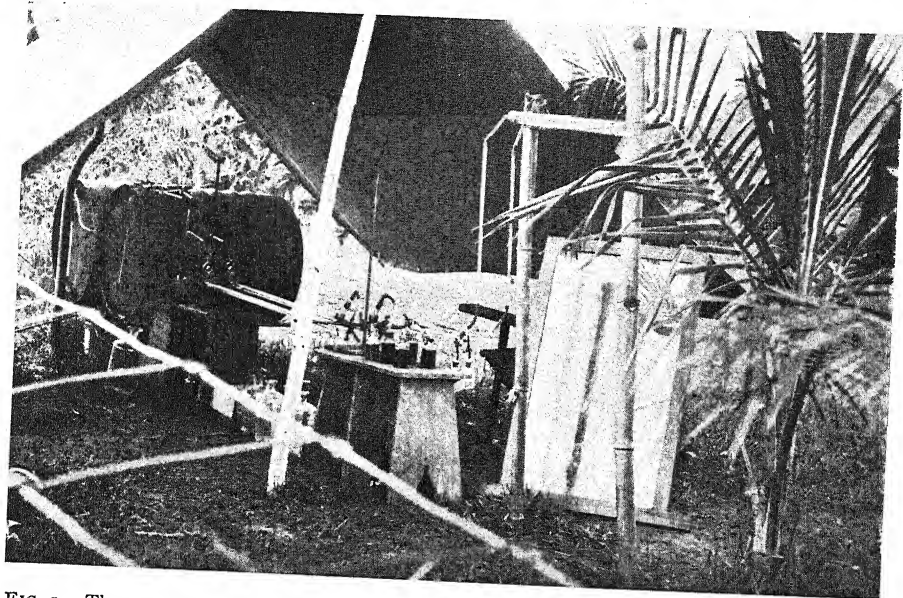


FIG. 1. The apparatus used in testing the carbon dioxide absorption by coco-nut, photographed from the SE. on May 28, at 5 p.m., during the test of leaf 4.

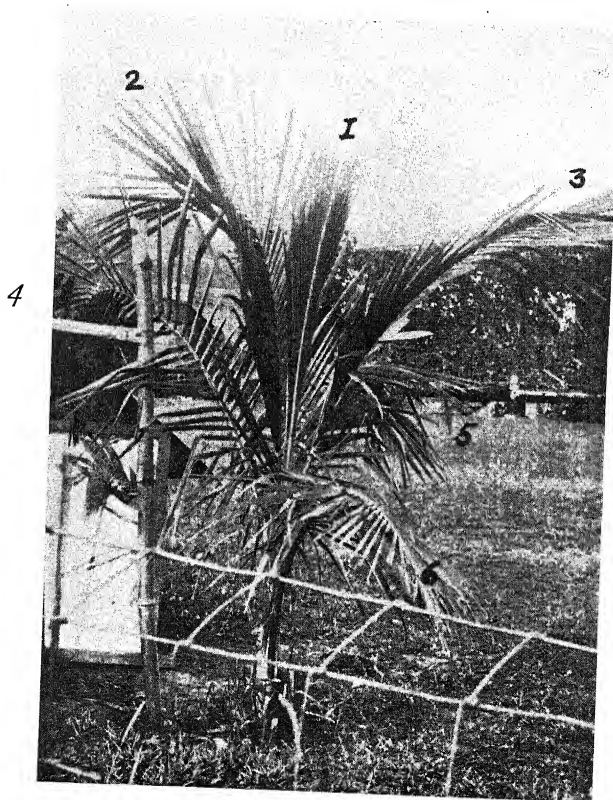


FIG. 2. A photograph of the coco-nut tree used in testing the carbon dioxide absorption of coco-nut leaves at different ages.



# The Mode of Infection by Smut in Sugar-cane.

BY

JEHANGIR FARDUNJI DASTUR, M.Sc.

*Supernumerary Mycologist, Pusa, India.*

With ten Figures in the Text.

SUGAR-CANE smut (*Ustilago Sacchari*, Rabenh.) is well known in almost all the cane-growing countries, but as yet the exact details of the manner in which sugar-cane is infected with smut have not been worked out.<sup>1</sup> Krüger<sup>2</sup> and Delacroix and Maublanc<sup>3</sup> believe that cane smut is not only produced by planting setts taken from infected parents, but also by direct infection of healthy plants. Wakker<sup>4</sup> has observed that setts taken from stools grown in the neighbourhood of smutted *Saccharum spontaneum* gave a diseased crop, and therefore Delacroix and Maublanc suppose that the spores infected the setts, probably the buds. In Java, artificial inoculations on cut surface of the setts and also on the buds have given diseased shoots in about a year. Ajrekar,<sup>5</sup> in Poona, succeeded in growing diseased shoots by planting setts after smearing them with spores. Dr. Butler, the Imperial Mycologist, in his unpublished notes, which he has very kindly allowed me to consult, says that he did not succeed in duplicating the results obtained in Java. He dipped, before planting, a hundred setts in water containing smut spores, and only one sett gave a smutted stool. My experiments show that the direct infection takes place only in two ways: (1) through very tender buds, (2) through old buds only when wounded. These results are in direct contradiction to those obtained in Java as far as the infection of setts through the cut ends is concerned. As regards direct infection there is discrepancy between the results obtained in Pusa, Java, and Poona, which perhaps can be explained in the light of the present work. It is possible that Dr. Butler, in Pusa, may have used setts from mature

<sup>1</sup> Butler, E. J.: Fungi and Disease in Plants, p. 378, 1918.

<sup>2</sup> Krüger, W.: Das Zuckerrohr und seine Kultur, p. 406, 1899.

<sup>3</sup> Delacroix, G., and Maublanc, A.: Maladies des plantes cultivées dans les pays chauds, p. 514, 1911.

<sup>4</sup> Wakker: Archief voor de Java-Suikerindustrie, p. 929, 1895.

<sup>5</sup> Ajrekar, S. L.: On the Mode of Infection and Prevention of the Smut Disease of Sugar-cane. Agr. Journ. India, xi, p. 292, 1916.

canes or back setts; these setts had therefore old buds, and so the results were negative. But in Java, for the experiments which gave positive results, setts from immature cane or top setts may have been used, and these setts had consequently tender eyes. The Poona results may be explained in the same way, but unfortunately Ajrekar's experiments are not convincing. From his Experiment No. 2 we find that healthy setts unsteeped (plot No. 2) gave 122 smutted shoots and diseased setts, unsteeped (plot No. 4) gave 228 smutted shoots. No explanation is given as to how healthy setts became smutted to such a large extent. Again, in Experiment No. 4, 25 setts (plot No. 2), first smeared with spores and then steeped in 1 per cent. copper sulphate solution, gave only 25 smutted shoots, while plot No. 3, a duplicate of plot No. 2 in all respects, gave over 500 smutted shoots. It is difficult to explain the disparity between the results of those two plots, and therefore the value of the whole experiment is vitiated; and consequently from his experiments it cannot be definitely concluded 'infection by spores adhering to setts takes place'.

#### INOCULATION EXPERIMENTS.

For the inoculations either fresh spores were used or cultures grown on bread paste. This medium has been found to be very suitable for germinating smut spores. In twenty-four hours a thin white film of mycelium containing innumerable sporidia are found where spores have been planted. The infection through sporidia takes place naturally more quickly than with spores.

Unless where otherwise stated, cuttings and plants of the susceptible 'thin' varieties, Seretha and Mungo, have been used for inoculations.

Inoculations on the cut ends of the setts and on the top of the crown, cut back without damaging the growing-point, have been unsuccessful. Several attempts have been made to infect the cut ends of the setts, but all have so far proved unsuccessful. These setts were kept in moist chambers.

Infection of rootlets and root buds of setts kept in moist chambers have also proved unsuccessful. Setts incubated in moist chambers have been successfully inoculated through tender buds, the scale leaves of which had not turned brown. In some cases the infection takes so readily that in four days' time the mycelium reaches the growing-point (Fig. 2). Old buds which have become swollen and the scale leaves of which are hard and brown could only be inoculated when wounded. The wound did not reach the growing-point, but only slightly exposed the inner tender pale green scale leaves. All these experiments were microscopically controlled. The control setts invariably remained healthy.

The inoculations of setts done under moist-chamber conditions were duplicated on potted plants.

Some of the experiments are detailed below.

If the bud to be inoculated was covered by the leaf-sheath, the latter was completely removed without injuring the bud. All the experiments had controls which invariably remained healthy.

Two very tender buds of a potted plant were inoculated by means of spores on June 22, 1918. About two months later, August 15, the shoots developing from these inoculated buds had produced the characteristic spore-bearing whip-like prolongations. Three days later the shoot from below the inoculated bud was also smutted. Microscopic examination of the plant showed that its tissues were filled with smut hyphae.

Plants inoculated through wounded roots and root buds remained healthy and no hyphae were found in their tissues.

The crowns of a few plants were cut back without injuring the growing-points and the tops of a few plants were completely removed, and the cut ends were inoculated with spores and with cultures on bread meal. All the infections failed.

On August 27, 1918, several plants were inoculated through tender wounded and unwounded buds. A month later sections were made of the small shoots arising from some of the inoculated buds. Smut hyphae were found in the growing-point and in the scale leaves. On November 6, one of the inoculated buds gave a spore-bearing shoot. After this date spore-bearing shoots were produced from time to time till April 1919.

One of the plants had all the inoculated buds except one removed for microscopic examination. The uninoculated buds below and above the inoculated buds gave smutted shoots. The whole cane was found to contain smut hyphae. Some of the adventitious roots arising from nodes above ground level were found to have their cortical tissues infected with smut hyphae.

One plant (Fig. 1) had only one bud (B) inoculated on August 27, 1918. This bud remained dormant till April 9, 1919, when it was sectioned and was found to contain smut hyphae in its tissues. The mycelium from the inoculated bud entered the node and travelled up and down the stem, and from the stem passed into the other buds. The smut hyphae in the main

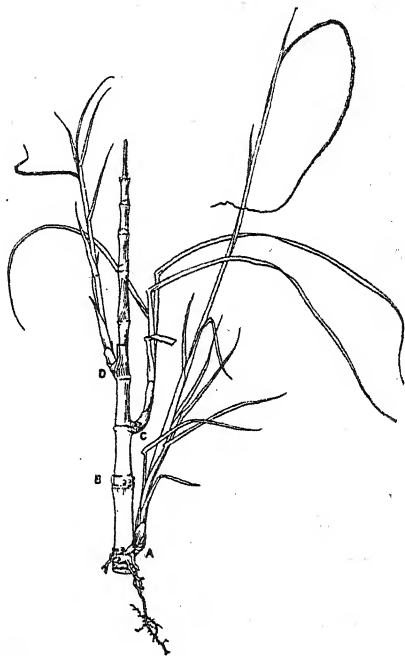


FIG. 1. Inoculated sugar-cane plant, for explanation see text.

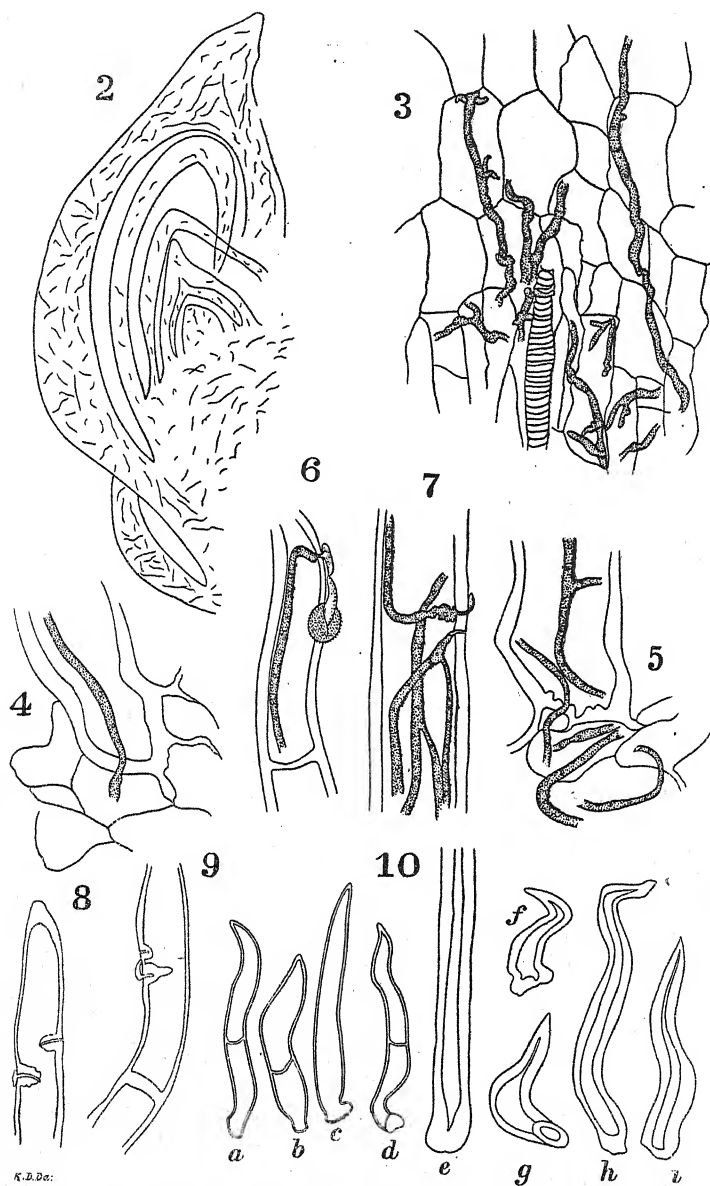
stem was traced right up to the growing-point. The bud (c) on the node immediately above the inoculated bud showed signs of sprouting in the beginning of January 1919, and it died in the end of March. No spore-bearing whip was produced. The bud (D) on the node above this dead shoot began to open in the middle of March and on April 10 developed a spore-bearing shoot. The bud (A) below the inoculated dormant bud was seen to burst in the beginning of March, and on April 6 the characteristic spore-bearing shoot was visible. Rootlets arising from this node and from the node below it had smut hyphae in their cortical tissues.

At times the inoculated bud dies before it has grown into a shoot. In this dead bud are found smut hyphae; they can also be traced into the node and in the internodes above and below this node. The extent of the penetration of the hyphae depends upon the time that elapsed since the inoculation.

A 'thick' variety of cane, Purple Mauritius, was inoculated on January 13, 1919, through wounded buds. Some of the shoots from the wounded bud were sectioned on April 26; smut hyphae were found in their tissues. Inoculations through unwounded young and old buds were unsuccessful. Another 'thick' variety, Sathi 131, was inoculated through wounded and unwounded tender buds on July 1, 1919, and the cuttings were incubated in moist chambers. On the 23rd, the shoots from all the wounded inoculated buds were found on microscopic examination to be infected, but the shoots from the unwounded inoculated buds showed no signs of infection.

On April 10, 1919, (1) unwounded old buds, (2) wounded old buds, and (3) unwounded tender buds were inoculated with cultures of the smut on bread paste. Five weeks later some of the buds from each of these series were sectioned and microscopically examined; smut hyphae were found in the inoculated wounded old buds and in the unwounded tender buds, but not in the unwounded old buds.

Some of the unwounded tender and wounded old buds inoculated in August and November 1918 remained dormant, and the plants did not show any external signs of infection. These plants were cut into setts and those setts which had the dormant inoculated buds were planted in five pots on March 28, 1919. On April 20, a smutted shoot was produced by one of these setts, the buds of which were inoculated without wounding them on November 28. Two setts, which had unwounded inoculated buds, gave smutted shoots on June 18 and September 7, 1919. A fourth sett, which had wounded inoculated buds, was found smutted on June 19, 1919, and the fifth sett developed a smutted shoot on November 15, 1919. One plant was inoculated through its buds by wounding them on January 13, 1919. The inoculated buds were dormant on April 28, when a cutting from this plant with the dormant inoculated buds was planted. On June 17, 1919, one of the shoots was found to produce a spore-bearing 'whip'.



K. D. Dastur:

FIG. 2. Longitudinal section of an inoculated leaf-bud showing the penetration of hyphae in four days.  $\times 51$ .

FIG. 3. Longitudinal section of a scale leaf.  $\times 357$ .

FIGS. 4 and 5. Hyphae in the hairs of a scale leaf.  $\times 368$ .

FIGS. 6-9. Penetration of hairs by the germ-tube from sporidia. Fig. 6,  $\times 550$ ; Figs. 7-9,  $\times 368$ .

FIG. 10. Hairs from scale leaves: *a-d*, thin-walled hairs which are capable of being infected; *e-i*, thick-walled hairs which cannot be infected.  $\times 245$ .



Having established the fact that infection can take place through unwounded tender buds and through wounded old buds, the next step was to find the exact place through which the infecting germ-tube enters the host tissues. It was suspected that infection takes place through unthickened scale hairs, because the cavities of the hairs of the inoculated eye-buds were found to be filled with unseptate or very sparsely septate hyphae, and in some cases germ-tubes from what looked like sporidia were found to have penetrated the hairs.

Ultimately a few cases were found which conclusively proved that infection takes place through the scale hairs. In one particular case a spore had germinated on the surface of a thin hair and from the end of the promycelium a sporidium had developed. From this sporidium, which was still attached to the promycelium, a fine germ-tube was developed, which pierced the thin wall of the hair. In the lumen of the hair the fine germ-tube broadened and travelled downwards (Fig. 6).

Other very clear cases (Figs. 7-9) of sporidia penetrating the hairs of the scale leaves by means of their germ-tubes have been observed from time to time. At times the hair reacts to the entrance of the germ-tube by developing a plug or thickening on the inner wall (Figs. 8 and 9). Whether the plug prevents infection is not definitely known. A few cases, however, have been observed in which the germ-tube was found beyond the plug.

It is only the unthickened hair that the sporidium is capable of infecting (Fig. 10, *a-d*). Hairs with thickened walls (Fig. 10, *e-i*) have not been found to be infected, though spores and sporidia have been found to be lying on them. So far the germ-tube from the sporidium has not been observed to enter directly the epidermal cells.

Inside the hair, the hyphae from the sporidium give out branches which at times completely fill the lumen of the hair. From the basal part of the hair the hyphae enter adjacent epidermal and sub-epidermal cells (Figs. 4 and 5). In the tissues of the scale leaves the mycelium is intracellular and consists of long strands of hyphae which are sparsely septate (Fig. 3). In the young stem of the leaf-bud the mycelium is at first both inter- and intra-cellular, but ultimately it is chiefly intercellular. The mycelium from the outer scale leaf travels downwards, enters the young stem of the leaf-bud through the leaf base, thence it travels up the inner leaves and the growing-point and also enters the main stem through the node. In some cases the hyphae in the outer scale leaf directly enter the inner scale leaf, the outer epidermis of which is closely adpressed to the inner epidermis of the outer scale leaf.

#### CONCLUSION.

The experiments conclusively prove that direct infection of 'thin' varieties of cane can take place through tender buds, but not through old

buds, unless wounded. 'Thick' varieties have been infected only through wounded eyes. So far as is known, the infection can take place only through unthickened scale hairs. The bud can produce a spore-bearing shoot within two months after infection. It is also evident that the hyphae from the infected bud, even when it remains dormant, travel into the main stem, thence to the tillers and secondary shoots, which ultimately may produce spore-bearing shoots. If from such a plant setts are used as 'seeds' before it produces spore-bearing shoots, which are the only visible sign of the presence of the disease, the new plants are bound to give infected stools, and setts containing inoculated buds which were dormant when planted have given smutted stools. These facts explain why setts taken from stools, which show no external sign of infection and look healthy in the absence of the spore-bearing shoots, give a new crop of smutted plants. It is therefore essential that setts used for inoculation experiments should be microscopically examined before they are inoculated.

#### SUMMARY.

It is shown that infection of the sugar-cane smut takes place only through the buds. The sporidia on germinating penetrate the young, thin-walled scale hairs. Infection through the cut ends of the setts does not take place.

A bud can produce a spore-bearing shoot within two months after infection. Diseased setts when planted give diseased shoots.



Choanephora cucurbitarum, (B. and Rav.) Thaxter,  
on Chillies (*Capsicum* spp.).

BY

JEHANGIR FARDUNJI DASTUR, M.Sc.,

*Supernumerary Mycologist, Pusa, India.*

With Plate XIX.

IN 1917 considerable damage was for the first time observed in chilli plants at Pusa and other chilli-growing tracts, north of the Ganges (India), by a new disease, caused by a species of *Choanephora*. In previous years it had not been found to be parasitic on this host, though it had been seen on fading flowers and dead twigs. In 1917 conditions of high humidity were generally prevalent in the last week of September and in the first week of October, when the plants had commenced to flower, and these conditions are believed to be correlated with the epidemic caused by this facultative parasite. It was first found on a few plants on September 30th, and was present in the fields till about the end of November. It was most virulent in about the second week of October. In 1918 careful search was made for this disease in Pusa and in the neighbouring villages, but not a single case was found. This may be due to the abnormally dry weather conditions prevalent in September and October.

The general appearance of the infected crop in the early stage of attack is not much unlike another disease, which does great damage in Behar, and which is caused by *Vermicularia capsici*, Syd., an account of which will be published later. There is the same drooping of the topmost tender parts of the plants and the dying back of the branches. But on a closer examination the effects of the *Choanephora* disease are very characteristic. The diseased parts show a distinct wet rot and are soon covered by a luxuriant crop of shining silvery conidiophores, without any trace of external vegetative mycelium. Unlike *Vermicularia capsici*, *Choanephora* attacks the leaves as well as stems; it does not produce on the infected stem the chalk-white areas, delimited by a black border, so characteristic of *V. capsici*.

The first evidence of the attack is generally when the flowers have opened; the infection starts from the flowers, as a rule, and sometimes also through the flower-buds. The flower first turns brown and then black, and

begins to rot. The rot then rapidly extends downwards, attacking buds and tender leaves. The affected tender parts of the stem hang limply. As the disease travels downwards the whole plant, in a severe case of attack, may be killed. The infected part of the stem shows a very marked wet green colour, and the bark easily peels off in shreds.

#### CHARACTERS OF THE FUNGUS.

*Conidial stage.* The conidiophores are erect and project at right angles to the epidermis. They are up to 5 mm. in length. They are whitish at first, but have a silvery metallic lustre when they are mature. They end in a capitate vesicle from which a few more ramuli arise, which in turn become vesicular (Fig. 1). These secondary conidiophores remain unbranched. From the capitella arise dense, black clusters of conidia, each seated on a short sterigma. Conidia are elliptical to oval in shape, finely striated, and light brown in colour (Fig. 2). The base of each conidium is provided with a hyaline appendage. The conidia measure  $13.2-24 \times 8.8-13.2 \mu$ .

The conidia germinate readily in water. Before germinating they increase considerably in size and become slightly lighter in colour. As a rule, a single lateral germ-tube is developed.

*Sporangial stage.* On the host in nature the conidial stage only is found, but the other stages, viz. sporangial, chlamyospore, and zygosporangium, have been developed in cultures. In fact, except the conidial stage, no other stage in the life-history of this genus has as yet been observed on the host plant under natural conditions. Cunningham<sup>1</sup> found that the sporangia of *C. infundibulifera*, (Curr.) Cunn., developed only when the fungus was cultivated under unfavourable conditions; Thaxter<sup>2</sup> failed to get the sporangia of *C. Americana*, which is the same as *C. cucurbitarum*, (B. and Rav.) Thaxter; but Wolf<sup>3</sup> succeeded in inducing them to form in cultures. The sporangia of the chilli *Choaneophora* have been developed not necessarily under unfavourable conditions of nutrition. They are formed in cultures always along with the conidia, though Wolf has found the sporangia of *C. cucurbitarum* apart from the conidia. Sporangia cease to develop when the fungus is grown for a long time in cultures. Like *C. infundibulifera*, the fungus also loses after a time its vigour of growth in artificial media.

Mature sporangia are evident as pendulous, large, black, globular bodies, but when young they are white in colour. They have big round columella (Fig. 5). The sporangial wall is smooth and colourless, the black

<sup>1</sup> Cunningham, D. D.: On the Occurrence of Fructification in the Mucorini, illustrated by *Choaneophora*. Trans. Linn. Soc., London, Ser. 2, Bot. i, 1879, p. 417.

<sup>2</sup> Thaxter, R.: A New English *Choaneophora*. Rhodora, v, No. 52, 1903, p. 101.

<sup>3</sup> Wolf, A.: A Squash Disease caused by *Choaneophora cucurbitarum*. Journ. Agr. Res., viii, No. 9, 1917, p. 323.

colour of the sporangia is due to the enclosed mass of brown spores. The sporangia vary a great deal in diameter, but usually they measure between 47.6 and 170  $\mu$ . The spores are of the same colour as the conidia, and they are of about the same size (16.5–24  $\times$  7.7–11  $\mu$ ), but the episporium is thicker and not striated. The spores have no hyaline appendage at the base, but they have a cluster of fine cilia at both ends (Fig. 6). They are equilateral, rarely three-cornered.

The spores germinate in the same way as do the conidia.

*Chlamydospores.* In cultures the hyphae at times develop intercalary vesicles. The vesicular walls either remain unthickened or, as in true chlamydospores, become thickened. The chlamydospores are highly vacuolar. Germinating spores also develop chlamydospores (Fig. 4).

*Zygospores.* The development of the sexual fructification of certain algae and aquatic fungi depends, according to Klebs,<sup>1</sup> primarily upon factors of nutrition and environment. Kauffmann<sup>2</sup> has found that the formation of oospores of some *Saprolegnia* can be influenced by definite chemical and physical conditions which can be readily controlled. Shear and Wood<sup>3</sup> believe that the perithecial-forming faculty in the numerous cases of *Glomerella*, studied by them, is evidently a fairly well fixed hereditary racial character, because if once a race or strain which produces perithecia in culture media is obtained other generations grown from this race or strain continues to produce perithecia indefinitely. Pethybridge and Murphy<sup>4</sup> have found the same racial hereditary character in the formation of the oospores of *Phytophthora infestans*; but in *Phytophthora parasitica* the author<sup>5</sup> has found that the oospore-producing faculty is an hereditary racial character only if each generation of the oospore-forming strain is grown on a medium different from the one on which the immediate parent was cultivated. But the formation of zygospores of the chilli *Choanephora* does not depend on the development of a strain which would in subsequent subcultures continue to give the sexual spores, nor upon other conditions mentioned above, but, curiously enough, the zygospores of this *Choanephora* are developed in cultures only when the mycelium arises from conidia taken directly from the host plant and not from the fungus growing on nutrient medium. This peculiarity in the production of zygospores has been observed by Wolf. The development of the sexual spores (Figs. 7–9) is similar to that of *C. infundibulifera* and *C. cucurbitarum*. The zyo-

<sup>1</sup> Klebs, G.: Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen, 1896.

<sup>2</sup> Kauffmann, C. H.: A Contribution to the Physiology of the Saprolegniaceae, with Special Reference to the Variations of the Sexual Organs. Ann. Bot., xxii, 1908, p. 377.

<sup>3</sup> Shear, C. L., and Wood, A. K.: Studies of Fungous Parasites belonging to the Genus *Glomerella*. U.S. Dept. of Agric., Bur. of Pl. Industry, Bul. No. 252, 1913, p. 72.

<sup>4</sup> Pethybridge, G. H., and Murphy, P. A.: On Pure Cultures of *Ph. infestans*, de Bary, and the Development of Oospores. Proc. Roy. Dub. Soc., xiii (N.S.), No. 36, 1913, p. 581.

<sup>5</sup> Dastur, J. F.: On *Phytophthora parasitica*, nov. spec. Mem. Dept. of Agr. India, Bot. Ser., v, No. 4, 1913, p. 204.

spores are more or less globular, with a thick, smooth, and brown episporium. They measure 56–88  $\mu$ .

Three species of *Choaneophora* have so far been known to be parasitic. Of these *C. Simsoni*, Cunningham,<sup>1</sup> on *Ipomoea rubro-coerulea* and *C. infundibulifera*, (Curr.) Cunningham,<sup>2</sup> on *Hibiscus* spp. and *Zinnia* spp. (synonymous with *C. Cunninghamiana*, Curr.), are Indian species, while *C. cucurbitarum*, (B. and Rav.) Thaxter,<sup>3</sup> which is the same as *C. Americana*, on *Cucurbita* spp., is American. The *Choaneophora* under study is distinct from its Indian allies. Not only in the smaller measurements of the sexual and asexual fructifications does *C. infundibulifera* disagree with the chilli fungus, but there is another very characteristic difference. In the *Choaneophora* on *Zinnia* the persistent portion of the capitella after the ripe conidia have become detached appear as a series of pedicellate funnels, from which the specific name, *infundibulifera*, is derived. This characteristic is wanting in the chilli fungus. Again, the sporangial wall of the former is conspicuously tuberculated, while that of the latter is smooth. *C. Simsoni* has smaller spores, conidia, and zygospores, but the most important difference is that its capitella are abruptly truncate, while those of the species here dealt with are dilated and globular.

The *Choaneophora* on chilli, however, resembles very closely the American species, *C. cucurbitarum* on *Cucurbita* spp. These two forms not only agree in their various spore measurements except those of the sporangiophores—the *Cucurbit Choaneophora* having slightly bigger sporangiospores (18–30  $\times$  10–15  $\mu$ )—but also in other characteristics. The capitella of both are rounded, the conidia have hyaline appendages at the base (these appendages are absent in the two Indian species), both have the same peculiarity in the development of the sexual spores, viz. that they are formed in cultures only when the conidia for making these cultures are taken direct from the host plant.

For these reasons the chilli parasite has been identified as *C. cucurbitarum*, (B. and Rav.) Thaxter.

#### SUMMARY.

A new disease of chillies caused by *C. cucurbitarum*, (B. and Rav.) Thaxter, is described.

It produces a wet rot and drooping of the infected tender parts and a dying back of the branches.

<sup>1</sup> Cunningham, D. D. : A New and Parasitic Species of *Choaneophora*. Ann. Bot. Gard., Calcutta, India, 1895, p. 169.

<sup>2</sup> Ibid. : On the Occurrence of Conidial Fructification in the Mucorini, illustrated by *Choaneophora*. Trans. Linn. Soc., Ser. 2, Bot. i, 1879, pp. 409–22.

<sup>3</sup> Thaxter, R. : loc. cit., p. 102.



The infection as a rule starts from the flowers, but sometimes also from the flower-buds.

Only conidia are developed in nature on the host, but sporangia, chlamydospores, and zygospores have been observed in cultures. The conidia bear a hyaline appendage at the base.

The sporangiospores have a cluster of appendages ('cilia') at each end. They are almost of the same size as the conidia.

The zygospores are developed in cultures only when the mycelium arises from conidia taken directly from the host plant.

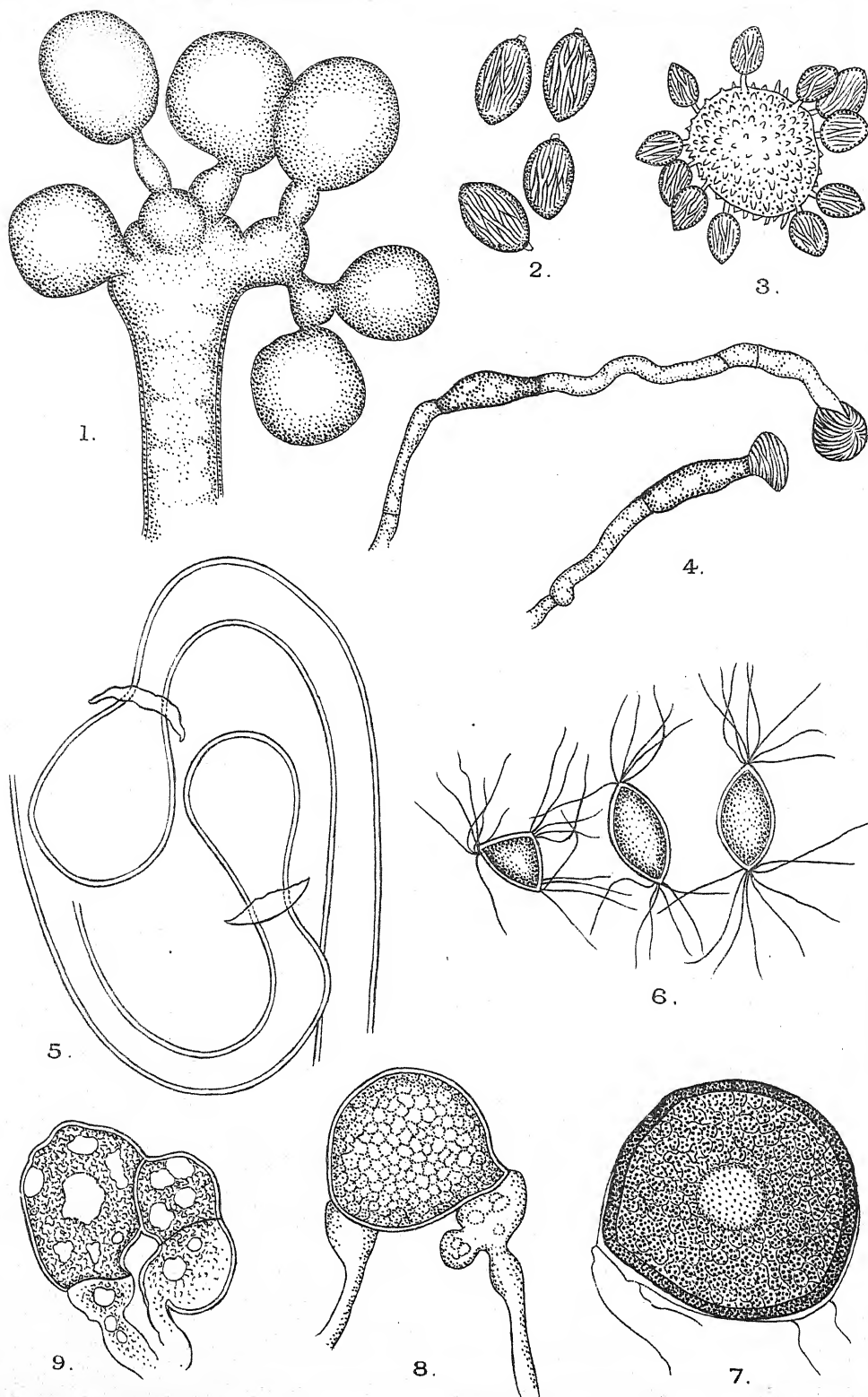
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### EXPLANATION OF PLATE XIX.

Illustrating Mr. Dastur's Paper on *Choanephora cucurbitarum*.

- Fig. 1. Conidial head with primary and secondary columella.  $\times 490$ .  
Fig. 2. Conidia.  $\times 710$ .  
Fig. 3. Surface view of a secondary columella bearing conidia.  $\times 490$ .  
Fig. 4. Germinating conidia.  $\times 490$ .  
Fig. 5. Sporangiphores with columella and remnants of the sporangial wall.  $\times 490$ .  
Fig. 6. Sporangiospores.  $\times 710$ .  
Fig. 7. Mature zygospore.  $\times 490$ .  
Fig. 8. Immature zygospore.  $\times 490$ .  
Fig. 9. An early stage in the development of the zygospore.  $\times 490$ .





J.F. Dastur del.

Huth, London.

Harold Welch P.

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# Osmotic Properties of some Plant Cells at Low Temperatures.

BY

FRANCIS J. LEWIS, D.Sc., F.R.S.E. & C., F.L.S.,

AND

GWYNETHE M. TUTTLE, M.Sc.,

*Botanical Department, University of Alberta, Edmonton, Canada.*

With five Figures in the Text.

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## INTRODUCTION.

DURING the last few years the cryoscopic method of determining the osmotic pressure of plant saps has largely replaced the older plasmolytic methods. Investigations have been directed to seasonal changes in the constitution of the sap, as well as to changes due to differences in habitat, both in the same species and in different species characteristic of various plant formations.

The most recent work dealing with the first aspect of the subject is the work published by Dixon and Atkins in the Proc. Royal Dublin Society (5). These authors have carried out a fine series of researches on the seasonal changes in osmotic pressure and the relative proportion of electrolytes and non-electrolytes in the cell sap, both in leaves and in the conducting tissues of stems and roots of various plants. This work was directed particularly to the elucidation of the part played by osmotic pressure in the ascent of sap. Attention was paid to methods of extracting the plant sap to ensure that the extract was a fair sample of the sap within the living cell—a matter to which but little attention had been paid by previous workers.

The ecological side of the problem has been attacked by Harris and Lawrence (1), working on the osmotic pressures of Jamaican rain-forest vegetation. These authors found that vegetation of different ecological habitats possessed different osmotic pressures, but no information is given in their work as to the relative proportion of electrolytes and non-electrolytes in the several types they investigated.

It seems probable that ability to withstand low temperatures depends entirely upon the organization and biochemical characters of the cells. Although attempts have been made to recognize modifications of structure which would enable aerial or subaerial organs to withstand low temperature, none have been found whose presence would render the plant resistant.

An increase in the concentration of the cell sap will lower the freezing-point, but this seldom amounts to more than  $2^{\circ}\text{C}$ . Müller (2) has shown that living plants usually have a lower freezing-point than the expressed sap. The fact that the actual freezing-point of the cell is lower is due to surface tension forces, such as capillarity and imbibition. Mousson (3) found that in a capillary tube of not more than 0.4 mm. diameter the freezing-point of water was lowered  $0.1^{\circ}$  to  $0.2^{\circ}\text{C}$ . In order that sub-cooling may take place a localized formation of ice must be avoided. When contact with ice is avoided sub-cooling is observed in water. Thus Dufour (4) obtained the sub-cooling of  $-12^{\circ}\text{C}$ . in small drops of water floating in a mixture of almond oil and chloroform.

In the experiments carried out by Müller-Thurgau, leaves were wrapped round the bulb of a sensitive thermometer and inserted in a freezing mixture with the thermometer scale projecting outside. With this method the temperature sinks until the maximum degree of sub-cooling is reached, and then rises to the true freezing-point, when ice formation takes place, after which it falls gradually. According to Pfeffer the sub-cooling in most plants is not more than  $-3^{\circ}$  to  $-4^{\circ}\text{C}$ . It is important to remember that most previous experiments have been performed with plants living in comparatively temperate climates, and results obtained in this investigation (in Western Canada) with plants subjected to long periods of  $-40^{\circ}$  to  $-50^{\circ}\text{F}$ . have some bearing on the question.

#### SCOPE OF WORK.

The scope of the present investigation may be indicated as follows :

1. To determine the osmotic pressure, relative amount of electrolytes and non-electrolytes, and sugars in a few typical plants living at low temperatures from the autumn throughout the winter.
2. To determine the temperature at which ice formation actually takes place in the living cells of the tissues.
3. To observe the condition of the cell contents at low temperature.

The materials chosen for investigation were *Picea canadensis* (Miller)

B.S.P., leaves; *Pyrola rotundifolia*, L., leaves; *Linnaea borealis*, L. var. *americana* (Forbes), leaves; *Populus tremuloides*, Michx., cortical tissues. During the winter some difficulty was experienced in collecting *Pyrola* and *Linnaea* from under the snow, thus limiting the number of observations that could be made with these plants.

#### METHODS.

According to the observation of Dixon and Atkins (5), sap expressed by simple pressure from living tissues does not give a fair sample of the sap in the vacuoles of the living cell. In the researches of these authors the protoplasm of the living cell was rendered permeable to all the solutes by the application of extreme cold, liquid air being used for this purpose. After freezing by this means the protoplasm is killed, rendered quite permeable, and the sap with all the solutes can be extracted by comparatively slight pressure.

As liquid air was not obtainable for these experiments, liquid CO<sub>2</sub> was used. This gives a temperature of  $-72^{\circ}\text{C.}$ , which results in the instant freezing of the tissues. The material was then placed in a glass vessel, rapidly thawed, and on treatment in a small screw-press about 25 c.c. of sap could be obtained from a comparatively small amount of material of all the plant tissues investigated. Care was taken to ensure that all the CO<sub>2</sub> was evaporated before the extraction of the sap. The increased ease with which the sap is extracted by this method is striking. One sample of untreated cortex of *Populus tremuloides* gave only a few drops of sap on pressing; an equivalent weight of cortex killed by freezing yielded 14 c.c. of sap with slight pressure.

The  $\Delta$  of the expressed sap was determined at once—usually within the hour—by the ordinary Beckman method, and the measurement of the electrical conductivity was then measured by the method of Kohlrausch. The estimation of sugars was made by treating the extracted sap with a minimum amount of basic lead acetate to precipitate the tannins. The excess of lead was then removed by sodium carbonate. The extract freed from tannins was tested with Benedict's sodium citrate solution (6). All determinations were made in drops rather than in cubic centimetres, since the quantity of extract available would not permit of the latter method. The same burette was used throughout the investigation, thus ensuring a uniformity in the size of the drops. The results recorded in each case were the number of drops required to decolorize twenty-five drops of Benedict's solution; the relative reducing power of the extract before and after inversion being determined by comparison with that of a standard glucose solution. The relative amount of glucose, sucrose, and maltose were determined according to the methods described by Haas and Hill (7).



To minimize any marked variation of the sugar content due to variation in photosynthesis at different times of the day, all material was collected at 2 p.m.

#### METEOROLOGICAL CONDITIONS.

Records of the temperature were kept from January onwards by means of a Casella Recording Thermograph. The instrument was fixed on a wall facing north, about sixty feet above the ground. The temperatures recorded were probably slightly above those obtaining at the level of the ground. A reduced record of the maximum and minimum temperature curve is given in Graph I. As the instrument only recorded down to  $+10^{\circ}\text{F}$ . the breaks in the diagram indicate periods below this temperature. The minima are recorded in figures in these blanks.

The first part of the winter was unusually free from low temperatures, the lowest temperatures coming in February and March.

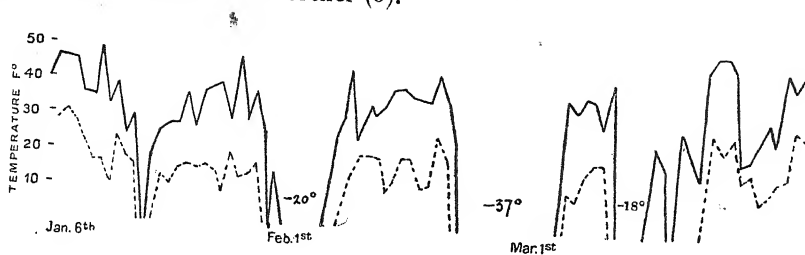
#### RECORDS OF OSMOTIC PRESSURES, &C.

All the material was obtained from the same habitat—a slope facing north. Trees of *Picea* of approximately the same age were used throughout—about fifteen years. To obviate any discrepancy due to individual differences of pressure in the trees, branches were cut from several trees, brought into the laboratory, and the leaves cut off. To eliminate any error due to differences in osmotic pressure of leaves of different ages only those from the previous year's shoot were taken. The leaves were then frozen and thawed in the manner already described and the cell sap expressed. The osmotic pressure was determined immediately, and then the conductivity, the whole process for one observation taking about three hours. The sugar estimation in some cases was made the following day, and in that case the sap remained frozen during the interval.

The depression of the freezing-point and the corresponding pressures in atmospheres are recorded in the tables and in the ordinates of the graphs. The figures in column C give the conductivity of the sap at  $37^{\circ}\text{C}$ . The figures under  $\Delta_e$  are calculated from those under  $\Delta$  and C, and represent the depression in freezing-point of solutions of potassium chloride having the same conductivity as those observed for the sap. They represent the total  $\Delta$  due to electrolytes. The column  $\Delta - \Delta_e$  represents the total  $\Delta$  due to the presence of non-electrolytes in the sap. This method was used by Dixon and Atkins (8), and gives clear indication of the main changes going on in the cell.

The material for the observations on *Populus*, *Linnaea*, and *Pyrola* was obtained from the same habitat as *Picea*. The respective readings are given in Tables I to IV and recorded in Graphs II to V. The atmospheric

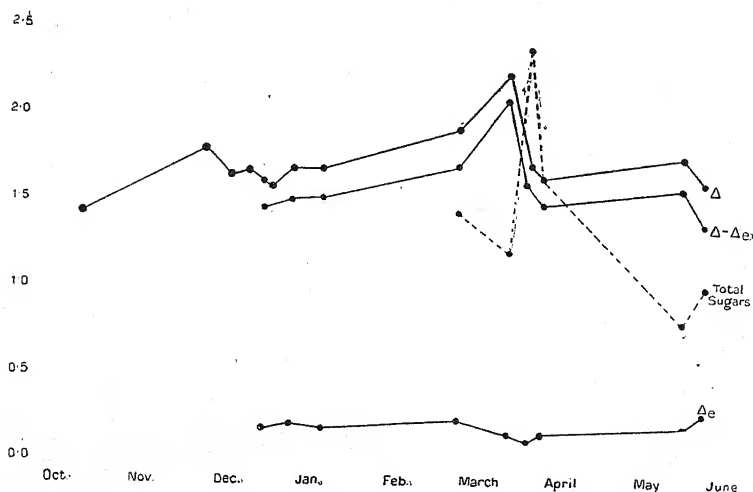
pressure P is the value corresponding to the observed  $\Delta$  given in the Tables published by Harris and Gortner (9).



GRAPH I.

TABLE I. *Picea canadensis*.

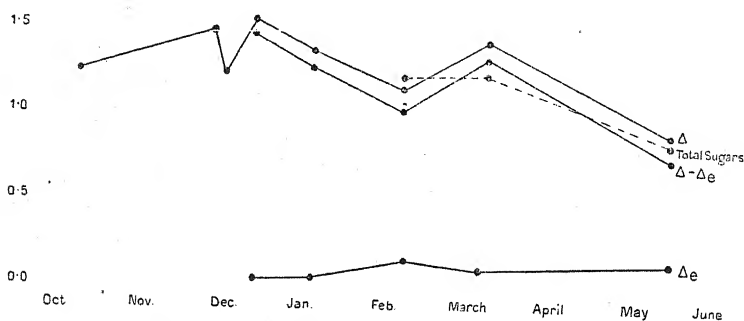
Date.	$\Delta$ .	P.	$\Delta_e$ .	$\Delta - \Delta_e$ .	$C \times 10^5$ .	Sucrose %.	Maltose %.	Glucose %.
Oct. 23	1.424	17.13	—	—	—	—	—	—
Dec. 7	1.777	21.36	—	—	—	—	—	—
" 15	1.655	19.91	—	—	—	—	—	—
" 22	1.680	20.20	—	—	—	—	—	—
" 28	1.635	19.66	—	—	—	—	—	—
Jan. 1	1.586	19.07	0.179	1.456	771	—	—	—
" 8	1.705	20.50	—	—	—	—	—	—
" 18	1.700	20.44	0.187	1.518	793	—	—	—
Mar. 7	1.909	22.94	0.179	1.521	771	—	—	—
" 26	2.240	26.91	0.214	1.695	921	0.20	0.97	0.24
April 2	1.720	20.68	0.143	2.097	617	0.16	0.58	0.47
" 7	1.625	19.53	0.119	1.601	514	0.39	2.00	0.01
May 26	1.750	21.04	0.142	1.483	612	0.62	0.00	1.00
June 4	1.635	19.66	0.191	1.559	822	0.00	0.77	0.03
			0.261	1.374	1121	0.00	0.00	1.00



GRAPH II. *Picea canadensis* (leaves).

TABLE II. *Populus tremuloides*.

Date.	$\Delta$ .	P.	$\Delta_e$ .	$\Delta - \Delta_e$ .	$C \times 10^6$ .	Sucrose %.	Maltose %.	Glucose %.
Oct. 22	1.247	14.99	—	—	—	—	—	—
Dec. 7	1.460	17.56	—	—	—	—	—	—
" 16	1.202	14.48	—	—	—	—	—	—
" 27	1.535	18.46	0.081	1.454	352	—	—	—
Jan. 18	1.344	16.17	0.083	1.261	354	—	—	—
Feb. 22	1.137	13.68	0.120	1.017	518	0.20	0.98	0.02
Mar. 23	1.410	16.96	0.078	1.332	335	0.20	0.98	0.02
May 28	0.877	10.56	0.114	0.763	493	0.08	0.28	0.45

GRAPH III. *Populus tremuloides* (bark and cortex).TABLE III. *Linnaea borealis*.

Date.	$\Delta$ .	P.	$\Delta_e$ .	$\Delta - \Delta_e$ .	$C \times 10^6$ .	Sucrose %.	Maltose %.	Glucose %.
Oct. 24	1.630	19.60	—	—	—	—	—	—
Dec. 26	2.080	24.99	0.342	1.738	1469	—	—	—
Mar. 21	2.127	25.55	0.343	1.784	1473	0.20	0.91	0.00
May 29	1.187	14.28	0.478	0.709	2056	0.00	0.00	0.31

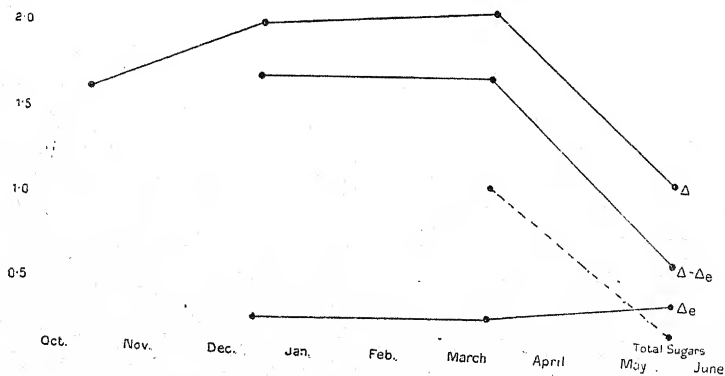
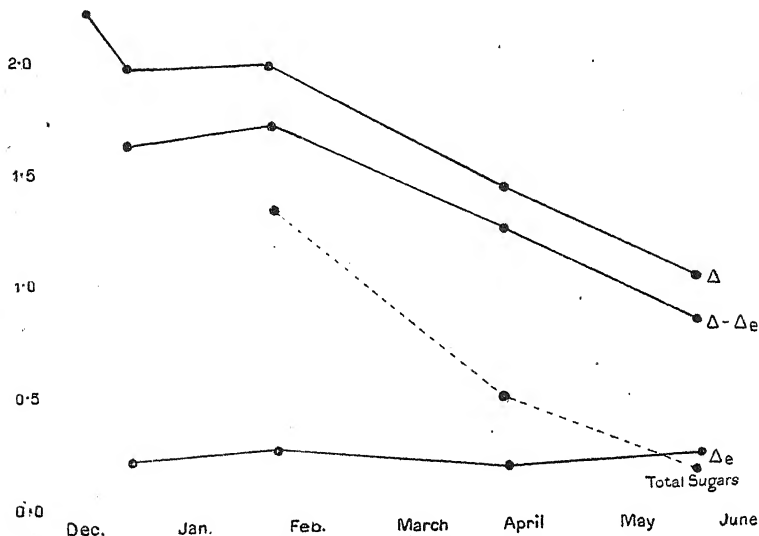
GRAPH IV. *Linnaea borealis* (leaves).

TABLE IV. *Pyrola rotundifolia*.

Date.	$\Delta$ .	P.	$\Delta_e$ .	$\Delta - \Delta_e$ .	$C \times 10^5$ .	Sucrose %.	Maltose %.	Glucose %
Dec. 19	2.222	26.69	—	—	—	—	—	—
" 29	1.863	22.42	0.223	1.640	986	—	—	—
Feb. 8	1.992	23.94	0.276	1.716	1186	0.12	1.21	0.00
April 11	1.430	17.20	0.181	1.249	780	0.00	0.11	0.37
June 4	1.045	12.58	0.239	0.806	1028	0.00	0.00	0.25

GRAPH V. *Pyrola rotundifolia* (leaves).AMOUNT OF UNDER-COOLING AND  $\Delta$  OF LEAVES.

Experiments were made to ascertain the temperature at which ice first appears in the leaves of *Picea*, *Linnaea*, and *Pyrola*. *Picea* leaves were found unsatisfactory to use for this purpose, as they could not be packed tightly round the bulb of the thermometer. *Linnaea* leaves are too small for successful use, and most of the observations were carried out with the leaves of *Pyrola*.

Experiments have been carried out by Müller-Thurgau on the freezing-point of cell sap *in situ* by either placing the bulb of the thermometer in a succulent tissue such as potato, or wrapping the bulb with the leaves to be tested. This method, while not yielding results comparable in accuracy to those obtained by placing the Beckman thermometer in a solution, is still of considerable value, as it is capable of indicating the amount of under-cooling which the minute volume of sap in the cell vacuole is capable of undergoing without the formation of ice. That the amount of sub-cooling in minute volumes, when contact with ice is prevented, may be much greater

than in comparatively large volumes is indicated by the work of Dufour (4). Direct observations on plant tissues have confirmed this. Thus Müller (2) found the lowest sub-cooling point without freezing of the grape was  $-6.8^{\circ}$  to  $-7.8^{\circ}$  C., while the real freezing-point of the sap was  $-3.1^{\circ}$  C. Dixon and Joly (10) found ice formation in the tracheides of *Taxus* began at  $-10^{\circ}$  to  $-11^{\circ}$  C. In our experiments we first carried out observations on greenhouse plants, and these are recorded in Table V.

TABLE V.

Material leaves.	Treatment.	Freezing mixture.	Maximum under-cooling.	$\Delta$ of sap within cells of leaf.
Impatiens	Fresh material from greenhouse	NaCl + Ice $-10^{\circ}$	$-1.3$	$-1.1$
Polypodium	" "	" "	$-2.9$	$-2.5$
Begonia	" "	" "	$-2.8$	$-2.1$
Syringa	" "	" "	$-2.8$	$-2.5$
Pyrola	Killed by freezing in $\text{CO}_2$ , thawed, and tested	Solid $\text{CO}_2$	$-3.5$	$-3.1$
Pyrola	Fresh leaves not previously killed	"	$-32.1$	$-31.65$

All these plants give a sub-cooling from  $-1.3^{\circ}$  C. in *Impatiens* to  $-2.9^{\circ}$  C. in *Polypodium* and the  $\Delta$  with formation of ice from  $-1.1^{\circ}$  C. in *Impatiens* to  $-2.5^{\circ}$  C. in *Polypodium*.

*Pyrola* leaves show very different values. The material was gathered outside during a spell of  $-20^{\circ}$  F. weather. A thermometer graduated to  $0.1^{\circ}$  and registering to  $-40^{\circ}$  C. was used in the Beckman apparatus, as the amount of under-cooling was too great to allow the use of the ordinary Beckman thermometer.

The behaviour of the living and dead leaves gave results of some interest. The leaves were killed by freezing in liquid  $\text{CO}_2$ , rapidly thawed, and the amount of under-cooling and  $\Delta$  determined. The fresh leaves under-cooled to  $-32.1^{\circ}$  C., rising to  $-31.65^{\circ}$  C. on the formation of ice in the tissues. The leaves previously killed with liquid  $\text{CO}_2$ , thawed, and tested in the Beckman apparatus using a freezing mixture of solid  $\text{CO}_2$ , under-cooled to  $-3.5^{\circ}$  C., rising to  $-3.1^{\circ}$  C. on the formation of ice in the tissues. Further observations will be carried out next winter by us using the leaves of other northern evergreens. That the condition of the cell contents during periods of low temperature determines the resistance of the tissue to cold is suggested in a paper by one of us in 1919 (11). *Linnaea* leaves naturally de-starchified are able to withstand the cold of a northern winter, whilst plants in which the fat has been reconverted into starch are quickly killed on exposure to temperatures of about  $-19^{\circ}$  F. ( $-28^{\circ}$  C.).

## CONDITION OF CELL CONTENTS AT LOW TEMPERATURES.

Pfeffer (12), in describing the work of Mohl, states that the brownish-green colour of evergreens during winter is due to a partial disorganization of the chloroplasts, accompanied by certain changes in the chlorophyll pigments. In some cases the browning only takes place when the cells are fatally affected, but with the restoration to normal conditions of light the chloroplasts in most conifers recover, turn green, and become functionally active.

During the progress of the present investigation we decided to examine the condition of the leaf cells of some of the material with a view to deter-

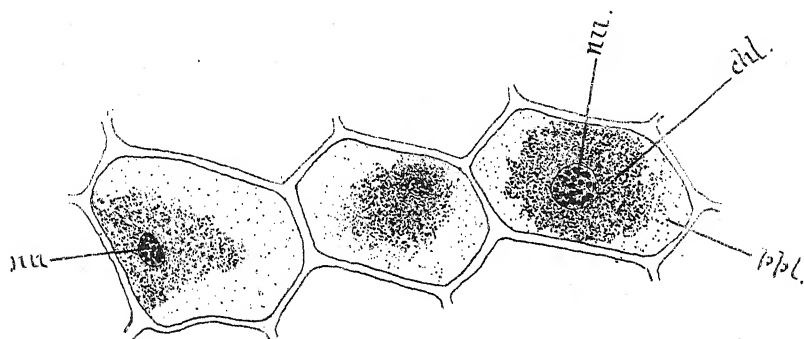


FIG. 1. *Picea canadensis*. Feb. 21. Mesophyll cells showing 'laking' of the chloroplast. Identity of individual chloroplasts completely lost, nuclei are prominent and very granular. *nu.* = nucleus, *ppl.* = protoplasm, *chl.* = chlorophyll.

mining whether similar features prevailed in this region. Microscopic examination of the mesophyll cells of *Picea* reveals a distinct localization of the cell contents during winter (Fig. 1). In this condition, which prevailed during the coldest weather, the identity of the individual chloroplast was completely lost. The diffuse chlorophyll mass, light yellowish green in colour, was segregated in a relatively small portion of the cell, closely associated with the very granular nucleus. The major portion of the cell was occupied by a large vacuole filled with fat (Fig. 2). All trace of starch disappears early in the autumn.

Leaves were examined frequently during the late winter and early spring, in order that the change to the summer condition might be observed. It was found that the change began quite early and took place within a short time. Cells examined on April 7 differed in several particulars from those of earlier preparations. The chlorophyll mass, though distinctly localized, gave evidence of the formation of distinct chloroplasts. Starch formation had commenced, but was localized in the region of the chloroplasts (Fig. 3). The central vacuole, though smaller, still gave a strong reaction when tested for fat with osmic acid. Three days later the change

had gone still farther; the chloroplasts, now definite in outline, were arranged at the periphery of the cell. Starch formation around the chloroplast was especially evident at this stage (Fig. 4). Material examined on April 14 revealed the cells in the normal summer condition, the chloroplast being entirely masked by the adherent starch granules (Fig. 5).

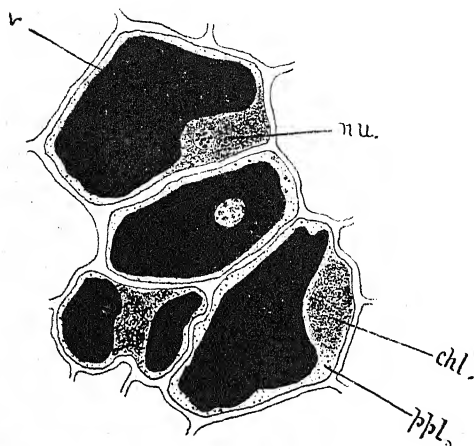


FIG. 2.

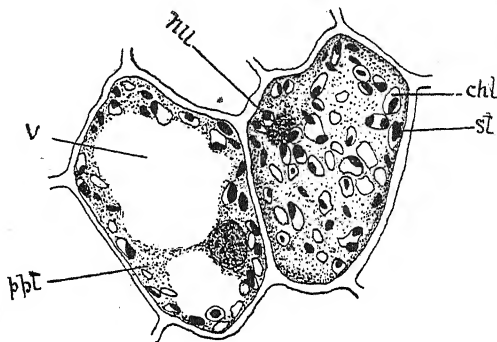


FIG. 4.

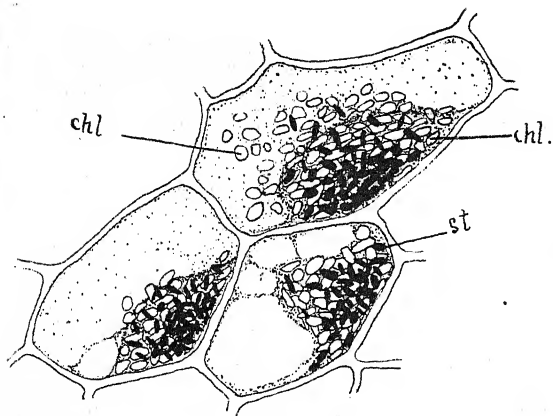


FIG. 3.

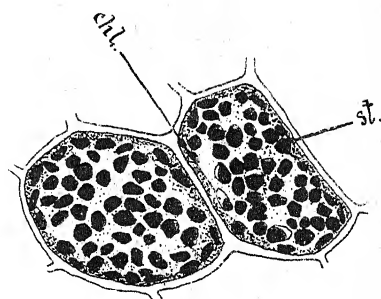


FIG. 5.

FIGS. 2-5. *Picea canadensis*. Fig. 2. Feb. 28. Mesophyll cells after treatment with 1% osmic acid showing vacuoles filled with fats. Fig. 3. Apr. 7. Mesophyll cells treated with iodine solution. Chloroplasts becoming distinct in outline although still localized in distribution; nuclei not visible; starch reaction localized. Fig. 4. Apr. 10. Mesophyll cells in iodine solution. Chloroplasts arranged at periphery of cell; nucleus visible and suspended in central vacuole; starch formation in region of chloroplasts. Fig. 5. Apr. 14. Mesophyll cells treated with iodine solution. Nucleus not visible at this stage. Chloroplasts closely packed round periphery of cell and masked by starch granules. *nu.* = nucleus, *v.* = vacuole, *chl.* = chlorophyll, *ppl.* = cytoplasm, *st.* = starch.



## DISCUSSION.

The changes recorded in osmotic pressures cannot be due to peculiarities of individual plants. In the case of *Picea*, material for each reading was collected from several different trees of approximately the same age, and in *Populus* the cortical tissue for each pressing was stripped from two or three young saplings. To obtain sufficient sap for a reading of *Pyrola* leaves from at least 50 or 60 plants had to be used, and more than that number were taken for *Linnaea*.

In *Picea canadensis* the osmotic pressure rose during the late autumn months, fluctuated slightly at the end of the year, and then rose steadily to a maximum in late March of 26.91 atmospheres. By the beginning of June the pressure had fallen to 19.66 atmospheres, but was still higher than the October value of 17.13 atmospheres. The electrolytes show only slight changes throughout the period from December to June, although a decided rise began to be apparent at the end; approximately 7 weeks after the end of severe frost. The variation of osmotic pressure appears to be due to non-electrolytes. The sugars do not appear to play the chief part in the variation of the non-electrolyte curve  $\Delta - \Delta_e$ , but the rapid increase in the total sugars—especially maltose—at the beginning of starch formation is noteworthy. The possible part played by the metabolism of fats in the variation of the osmotic curve will be discussed in a future paper.

In *Populus tremuloides* two maxima are observed in the osmotic pressure, but, as in *Picea*, the pressure falls decidedly as the summer condition is reached. The difference between the maximum and minimum osmotic pressure is only about one-half what it is in the leaves of *Picea*. Here again, the variation in pressure is due chiefly to non-electrolytes, although the electrolytes show a slight increase from March onwards.

In *Linnaea borealis* the maximum osmotic pressure is attained about the third week in March and falls rapidly until June, and the electrolyte curve shows a decided rise at the end of the observations. It was impossible to avoid using a number of young leaves for the reading on May 29, and the small osmotic pressure and increase in the electrolytes may be due to this.

*Pyrola rotundifolia*, unlike the other examples, shows a fairly steady decrease in osmotic pressure from December to June, and the electrolytes remain fairly constant throughout the season. The same fall in the sugar content occurs as the spring is approached, but commences somewhat earlier than in the other plants.

## SUMMARY.

1. Osmotic pressures, electrical conductivities, proportions of electrolytes and non-electrolytes, and the amounts of sucrose, maltose, and glucose have been determined in the leaf tissues of *Picea canadensis*, *Linnaea borealis*, *Pyrola rotundifolia*, and the cortical tissues of *Populus tremuloides* at intervals from the autumn until the summer.

2. No certain correlation between the above values and the daily or weekly fluctuations of air temperatures can be recognized without observations extending over more than one season.

3. The maximum osmotic pressure is reached in *Picea* and *Linnaea* towards the end of March; in December in the case of *Populus*, although a second maximum occurs in late March nearly of the same value as the December reading. *Pyrola* shows a fairly steady decrease from the middle of December till June.

4. The concentration of electrolytes shows very little variation in any of the plants during the whole period of observation.

5. The variations in osmotic pressure are due chiefly to the non-electrolytes.

6. The variation of the sugar content closely follows the variation of the osmotic pressure.

7. The sugars show a decided concentration during the winter months.

8. There is a progressive decrease of the sugars from the winter maximum towards the summer.

9. Leaves of *Pyrola* killed by freezing in liquid  $\text{CO}_2$  show ice formation at  $-3.1^\circ\text{C}$ .

10. In the living leaves of *Pyrola* ice formation does not begin until a temperature of  $-31.6^\circ\text{C}$ . is attained.

11. During the winter months chlorophyll granules in *Picea* are completely laked, the chlorophyll being localized in the region of the nucleus.

12. During the early part of April the chloroplastids assume definite form and starch formation commences.

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# Studies in Seed Germination.

## Experiments with *Cyclamen*.<sup>1</sup>

BY

ARTHUR W. HILL, F.R.S.

With Plate XX and fifteen Figures in the Text.

THE question as to whether the seedling of *Cyclamen* is 'monocotyledonous' or 'dicotyledonous' has attracted the attention of numerous botanists, and up to the present may still be regarded as being an open one. Bernhardt<sup>2</sup> in 1832 made the suggestion that the single seed-leaf of *Cyclamen* represents two fused leaves, basing it on the analogy of the tube formed by the two cotyledons of *Dodecatheon*; Lord Avebury also observed that abnormal seedlings of *Cyclamen* may have divided leaf-blades;<sup>3</sup> but neither of these views need be seriously considered as tending to prove that the single cotyledon of *Cyclamen* is really a double structure.

The late Miss Sargent<sup>4</sup> remarked that the 'vascular structure' of *Cyclamen persicum* 'suggests very strongly that the cotyledonary member consists of two seed-leaves united into a solid tube', but, she added, 'until the structure of allied genera has been worked out no great weight can be attached to this observation'.

The main purpose of the present paper is to give an account of some experiments and observations made on several species of *Cyclamen*, which, it is considered, afford definite proof that the embryo of *Cyclamen* does possess two cotyledons, only one of which, however, under normal conditions, develops into a leafy structure.

The opinions held by the earlier botanists on the nature of the *Cyclamen* seedling have been so well summarized by Gressner<sup>5</sup> that they need no further reference here. Notice, however, must be taken of

<sup>1</sup> Read before the Linnean Soc., June 6, 1918. See Proc. Linn. Soc., June 6, 1918.

<sup>2</sup> Bernhardt: *Linnaea*, vii, 1832, p. 578.

<sup>3</sup> Lubbock, Sir J.: A Contribution to our Knowledge of Seedlings, ii, 1892, p. 184. The double-leaved seedlings were no doubt specimens in which the original lamina had been injured and had been replaced by two new laminae produced from the top of the petiole.

<sup>4</sup> Sargent, E.: A Theory of the Origin of Monocotyledons founded on the Structure of their Seedlings. See *Ann. Bot.*, xvii, 1903, p. 76.

<sup>5</sup> Gressner, H.: *Bot. Zeit.*, 1874, p. 801, with plate.

Gressner's own view (l. c., p. 837), which is that *Cyclamen* has two cotyledons. The second cotyledon, according to him, is present only as a rudiment in the mature embryo, but develops later to form the second green leaf of the plant. Hildebrand's<sup>1</sup> views are based on more sound observation, for he speaks of the 'second leaf' as a small protuberance and mentions that its development can be artificially hastened by the removal of the blade of the 'first' leaf (i. e. the cotyledon). But both Gressner and Hildebrand confuse the issue by referring to the 'second' leaf, for under normal conditions the embryonic rudiment or protuberance very rarely develops, and the 'second' leaf of the young seedling *Cyclamen* is actually the first plumular leaf and is similar both morphologically and physiologically to the mature leaves of the plant.

Hildebrand, like Gressner, does not distinguish clearly between the true second cotyledon and the first plumular leaf, and judging from his concluding paragraph (l. c., pp. 96, 97) he did not consider the question of any particular interest in the biology of the seedling of *Cyclamen persicum*.

The embryo of *Cyclamen persicum* is also described and figured by Schmid.<sup>2</sup> He points out that there are two cotyledons, one of which is represented only by a rudiment, but he does not follow the germination or study the fate of the rudimentary second cotyledon.

Coulter and Chamberlain,<sup>3</sup> in their discussion of dicotyledonous embryos with a single cotyledon, are thus incorrect in stating that Schmid 'found embryos in ripe seeds of *Cyclamen persicum* with no trace of a second cotyledon'. These authors point out that in certain dicotyledonous forms there may be early abortion which may even approach suppression of one of the cotyledons; and that in consequence of this the single functional cotyledon may appear terminal and the stem-tip lateral. 'To call such cases "pseudomonocotyledons", however, is not consistent with the real nature of the monocotyledonous embryo.'<sup>4</sup>

No definite pronouncement is made by Goebel<sup>5</sup> as to whether *cyclamen* possesses two cotyledons or only a single one. He speaks of the seedling having only a single leaf, which is followed in due course by other primary leaves of similar form, and allows it rather to be assumed that there is only a single cotyledon in this genus.

Goebel<sup>6</sup> carried out an extensive series of experiments on *Cyclamen*

<sup>1</sup> Hildebrand, F.: Die Gattung *Cyclamen*. Jena, 1898, p. 95.

<sup>2</sup> Schmid, B.: Bot. Zeit., 1902, p. 217, Pl. IX, Figs. 45-7. The lettering given in the explanation of Fig. 47, which shows the second cotyledon rudiment, does not agree with that on the plate.

<sup>3</sup> Coulter and Chamberlain: Morphology of Angiosperms, 1904, p. 206.

<sup>4</sup> Ibid., p. 207.

<sup>5</sup> Goebel: Einleitung in die experimentelle Morphologie der Pflanzen, 1908, p. 203.

<sup>6</sup> Goebel: Ueber Regeneration im Pflanzenreich. Biolog. Centralbl., xxii, 1902, pp. 435-8 and pp. 481-7, with numerous text-figures. This work is shortly summarized in his Einleitung in die experimentelle Morphologie der Pflanzen, 1908, pp. 203-6.

*persicum* in connexion with the regeneration of the lamina from the cotyledonary petiole. His interpretation of the *Cyclamen* seedling is not very clear, but he definitely states that the genus differs from other Dicotyledons in *not* possessing two cotyledons (l. c., p. 435), but only a single seed-leaf.

It is on this single seed-leaf or cotyledon that Goebel carried out his regeneration experiments, and he makes no reference to the development of a second cotyledon. He states, however, that in all his experiments he removed 'the second leaf', but whether this second-appearing leaf was actually the second cotyledon or, more probably, the first plumular leaf is not certain. He refers to the leaves which arise after the cotyledon as the first leaves, 'Primärblätter', whatever their morphological nature may be, and there is no evidence that he distinguished between the *true* second cotyledon and the subsequent plumular leaves.

In the course of this paper I have pointed out that the regeneration of a lamina from the petiole is a peculiarity possessed by the cotyledonary petioles only, and Goebel also notes (p. 438) that he was unable to obtain regeneration with the older leaves of plants of flowering age. He appears to state, however, in a sentence,<sup>1</sup> the exact meaning of which is somewhat obscure, that the leaves following the 'single' cotyledon have similar powers of regeneration to that organ. These leaves, which he calls the 'Primärblätter', should be the plumular leaves, and with such I find no regeneration of the lamina takes place. Possibly he may have experimented with the *second leaf* of a seedling which did happen to be a true second cotyledon, otherwise I am unable to reconcile his conclusion.

Winkler<sup>2</sup> also, it may be mentioned, shows no particular interest in the question of the monocotyledonous or dicotyledonous character of the *Cyclamen* seedling, since his experiments are concerned with the behaviour of the first seed-leaf under traumatic stimulation, but it may be inferred from his account that he considers *Cyclamen* to be possessed of only a single cotyledon.

A preliminary account of my own experiments with reference to the development of the second cotyledon of *Cyclamen* was given at the meeting of the British Association at York in 1906.<sup>3</sup>

The *Cyclamen* seedling, as is well known, consists of a short hypocotyl—the young tuber or corm—bearing at its apex two organs placed exactly

<sup>1</sup> p. 438: 'Bei den Regenerationsversuchen, die oben kurz geschildert wurden, entfernte ich das zweite Blatt meist sobald es sichtbar war, übrigens verhalten sich die dem Kotyledon folgenden Blätter betreffs ihres Regenerationsvermögens ebenso wie diese' (*sic*).

<sup>2</sup> H. Winkler: Ueber die Regeneration der Blattspreite bei einigen Cyclamenarten. Ber. d. Deut. Bot. Gesellsch., xx, 1902, p. 82.

<sup>3</sup> In the course of my experiments, which were commenced at Cambridge in 1904, I have examined the seedlings of *Cyclamen Atkinsi*, *C. balearicum*, *C. Coum*, *C. libanoticum*, *C. neapolitanum*, and *C. persicum*. Figures of the seedlings of several species of *Cyclamen* are given by Hildebrand, l. c. See A. W. Hill: The Seedlings of certain Pseudo-monocotyledons. Report Brit. Assoc. York, 1906, p. 763.

opposite one another. One of these, which has already functioned as the absorbent organ in the seed, quickly develops its lamina and becomes an assimilating leaf, while the other remains in a rudimentary condition and is a scarcely visible, curved protuberance. The single assimilating organ or cotyledonary lamina is usually more or less cordate in outline with a well-marked notched apex, and is borne on a stout petiole. The cotyledonary petiole is slightly concavo- or plano-convex, a shallow groove or furrow being noticeable on the flattened inner or adaxial side, and it is from the edges of this groove—the adaxial ridges—that new laminae may be regenerated. Under normal conditions the *Cyclamen* seedling remains in



TEXT-FIG. 1. *Cyclamen neapolitanum*. A germinating seed, showing the cotyledon petiole, c. 1, and the rudiment of the second cotyledon, c. 2.

this one-leaf stage for some time and the other rudimentary organ (the second cotyledon) ultimately shrivels and dies. When a second green leaf does develop under normal conditions in due course, it is seen to differ from the seed-leaf in the shape of its lamina and in the character of its margin and markings. Moreover this second leaf does not arise opposite to the seed-leaf, the rudimentary aborted organ being in that position, but is placed between and at right angles to the two embryonic structures. This second leaf which springs from the plumular axis is thus seen to be the first leaf of the plumule or shoot. The suppression of the short axis, owing to the geophilous habit of the genus and the consequent crowding of the developing plumular leaves, may have led to the divergent views which have

found expression as to the morphological nature of the *Cyclamen* seedling.

The seedling at this stage may be compared to that of *Abronia* (Nyctagineaceae), where in the embryo and on germination only one cotyledon is in evidence, the other being represented by a minute and very slightly developed organ.<sup>1</sup>

The evidence as to the morphological nature of the second green leaf of *Cyclamen* afforded by its position receives some support when the anatomical structure of its petiole is compared with that of the cotyledons.

Even then doubts have been expressed as to the true character of this normally developed second leaf; but these, it is hoped, may now be considered to be finally settled by the regeneration experiments on cotyledonary and plumular leaves described in the following pages.

The development of the rudimentary curved organ at the apex of the hypocotyl—the rudiment of the second cotyledon—may be induced, as

<sup>1</sup> See Lubbock: On Seedlings, vol. i, p. 31, Fig. 64; he figures the embryos of *Abronia arenaria* and *A. umbellata*. According to Jepson, Gray, and Brewer, Botany of California, vol. ii, p. 3, the embryo in the genus *Abronia* is 'by abortion monocotyledonous'. In *Allionia*, the next genus, the embryo is described as being plicate, the inner cotyledon being shorter than the outer.

Hildebrand has pointed out, by removing the lamina of the seed-leaf of the young seedling. A more certain way of stimulating the rudimentary second cotyledon to develop, however, is to remove not only the blade but also the greater part of the petiole of the original seed-leaf. The second cotyledon is also frequently stimulated to develop if by any chance the lamina of the first cotyledon should be unable to escape from the seed-coats and so fail to function as an assimilating organ.

The second cotyledon is, as a rule, a smaller organ than the first, but otherwise it is very similar in general appearance; the apical notch so characteristic of the seed-leaf is usually noticeable, and the margins tend to be smooth and are not as a rule provided with the serrations which are characteristic of the margins of the plumular leaves. Conspicuous surface markings, which are a feature of the plumular leaves of *Cyclamen*, are also absent from the laminae of both cotyledons.

The vascular structure of the cotyledonary petioles shows some difference from that of the petiole of the young plumular leaves, but the differences are not constant. In the plumular leaf-petioles, especially near the lamina, there is usually a definite reniform stele surrounded by a distinct pericycle and endodermis and enclosing some three to seven radiating xylem groups of equal size.

In both the cotyledonary petioles, however, the vascular structure, as Miss Sargent noted for the first cotyledon, suggests a double structure. The xylem elements are arranged in two well-developed lateral groups, especially near the base of the petiole, while near the apex a somewhat feeble median xylem group may be seen in cross-section. The double type of bundle, which to some extent seems to be associated with a slender petiole and a small lamina, has also been noticed in sections of the petioles of adventitious leaves arising from the decapitated tuber.<sup>1</sup>

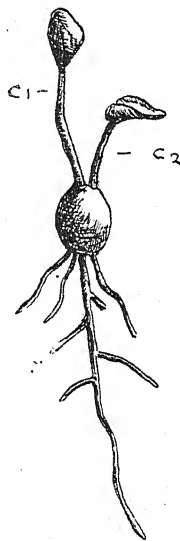
<sup>1</sup> Mr. Boodle has kindly examined sections of the petioles, and has made the following observations:

'In the double bundle there are sometimes two protoxylem groups, one to each xylem mass, or sometimes there are three protoxylems, two to the larger group of xylem, one to the smaller. The latter case gives an indication of the triple structure attained higher in the petiole by division of the larger xylem group, accompanied by displacement whereby one of these groups takes up a median position.

'Where the base of the petiole has a triple bundle there may be three protoxylems, but here,



TEXT-FIG. 2. *C. neapolitanum*. The cotyledonary lamina with the seed have been removed, and the second cotyledon, c. 2, is developing. The petiole of the normal cotyledon, c. 1, is beginning to wither.



TEXT-FIG. 3. *C. neapolitanum*. The first cotyledon, c. 1, has been unable to free its lamina from the seed-coat, and the second cotyledon, c. 2, has developed and expanded its lamina.



Passing now to the behaviour of the cotyledonary and plumular leaves when subjected to artificial mutilation, some striking differences are noticeable, as Winkler and Goebel<sup>1</sup> have pointed out.

If the lamina of the first cotyledon be removed, there will arise, from one or from both the ridges on the inner side of the petiole, just below the cut surface, small protuberances which will gradually develop into new laminae.

The new laminae grow out from the petiole as decurrent wing-like organs, and like the lamina are bifacial with their upper surfaces facing

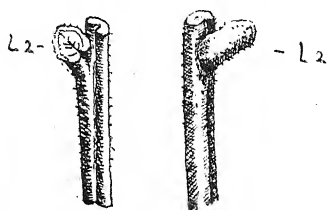


FIG. 4.

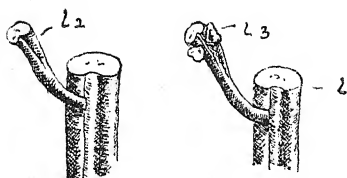


FIG. 6.

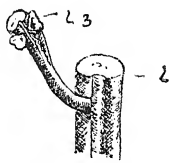


FIG. 7.

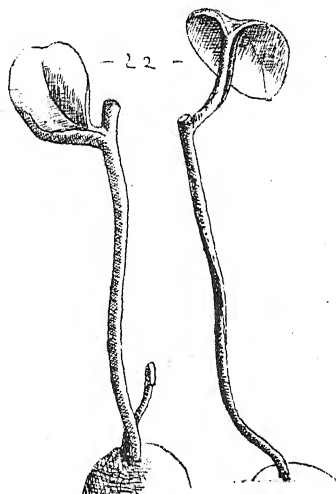


FIG. 5.

TEXT-FIGS. 4-7. 4. *C. persicum*. The petiole of the normal cotyledon with the lamina removed. A new lamina, *L 2*, has developed from the edge of the groove of the petiole near the cut surface. Back and front views. 5. *C. balearicum*. The formation of new laminae, *L 2*, from the cotyledonary petiole. 6. *C. persicum*. The new lamina, *L 2*, has developed a petiole and the lamina has been removed. 7. The development of new laminae, *L 3*, from the secondary petiole.

inwards. These new laminae are thus in their position at right angles to the original lamina of the cotyledon. Sometimes they remain as longitudinal wings or flanges attached by a broad base to the upper part of the petiole, but more often they assume the form of small leaves and develop distinct petioles of their own.

If after a time the upper part of the original petiole with the new laminae be removed, the petiole will again produce new laminae just below the cut surface, and it has been found that even if as much as 6 mm. of the apical portion of the cotyledonary petiole be removed new laminae will be

and also in double bundles, the protoxylems may be more or less united, so that an apparently single, rather diffuse, protoxylem group may occur on the adaxial side of the bundle.<sup>2</sup>

<sup>1</sup> Winkler: Ueber die Regeneration der Blattspreite bei einigen Cyclamenarten. Ber. d. Deut. Bot. Gesellsch., xx, 1902, p. 81.

Goebel: l. c., 1902, pp. 436-8, Figs. 10-13; and l. c., 1908, p. 203, Fig. 105.

Similar experiments were made by me at Cambridge in 1907 and 1908 on *Cyclamen balearicum* and *C. neapolitanum* independently of Winkler's and Goebel's work.

developed from the top of the decapitated petiole. Similarly, if one of the new lateral laminae be removed from its petiole, this secondary petiole is capable of producing a new lamina in the same way as does the main petiole of the cotyledon.

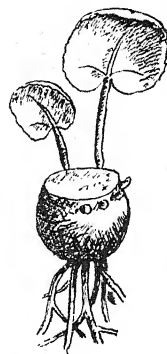
I have also been able to confirm Goebel's experiments with the hypocotyl of the *Cyclamen* seedling;<sup>1</sup> for if the top of the tuber with its plumule be removed, a series of adventitious leaves with petioles are formed round the periphery of the mutilated tuber from the cut surface (Pl. XX, Figs. 4 and 7, and Text-Fig. 8). The new leaves, in the cases which have been observed, arise with their outer or lower surfaces directed towards the centre of the tuber, and not, as figured by Goebel, with their inner or upper surfaces facing inwards. As they develop, however, they sometimes change their position and face the centre of the tuber. The decapitated upper portion of the tuber bearing the plumule will also send out adventitious roots from the cut surface—i.e. the lower surface—and behave as a true cutting.

The power of regeneration displayed by the hypocotyl is well known in some other plants, as for instance *Anagallis coerulea* and *Linaria cymbalaria*, in which, if the cotyledons and plumule be removed, new buds are formed freely on the hypocotyl.<sup>2</sup> A similar development of hypocotyledonary shoots also occurs in *Linum usitatissimum*, *Linaria bipartita*, and *Antirrhinum majus* on decapitation of the seedlings.<sup>3</sup>

The hypocotyl of *Cyclamen* appears to differ at first from the cases just mentioned, since for about a year laminae only are produced from the edge of the cut surface, and there are no signs of the formation of new growing-points from the tissues of the decapitated tubers. In the case of older tubers, as Goebel points out, adventitious shoots will also arise when the tubers are decapitated, but the new laminae and growing-points arise from the actual cut surface near the centre of the tuber instead of from the margin (Pl. XX, Fig. 6).

A further example of limited meristematic activity is shown by the cotyledon itself when that organ with its petiole is severed from the hypocotyl and treated as a 'cutting' (Pl. XX, Fig. 2).

In the course of a few days, under suitable conditions, roots are freely



TEXT-FIG. 8. A seedling of *C. persicum* from which the top of the tuberous hypocotyl with the cotyledon has been removed. Adventitious leaves arise from the tuber just below the cut surface. The specimen shows four rudimentary and the backs of two fully developed leaves.

<sup>1</sup> Goebel: l. c., 1902, pp. 482-4, Figs. 14, 15; and l. c., 1908, p. 204, Fig. 106.

<sup>2</sup> Küster: Beobachtungen über Regenerationserscheinungen an Pflanzen. Bot. Centralbl. Beihefte, xiv, 1903, p. 316.

<sup>3</sup> Burns and Hedden: Conditions influencing Regeneration of Hypocotyl. Bot. Centralbl. Beihefte, xix, 1906, p. 383.

produced from the base of the petiole and grow vigorously, being supplied with carbohydrates by the green lamina.

These leaf-cuttings will live for months, both lamina and stalk increasing considerably in size, but though numerous experiments were made at various seasons, it appeared that the tissues had no power to develop a growing-point and in due course the rooted leaf died.

A series of *Cyclamen* cotyledons were, however, put in as cuttings in October, 1918, in order to see whether any further stages in development would take place, and somewhat unexpected results were obtained. The petioles produced roots from the base readily as before, and in the following February (1919) a second leaf was found to be developing. On examination it was seen that a small tuber had formed at the base of the petiole as a lateral outgrowth, and it was from this tuber that the second leaf, which was plumular in character, had arisen. Nearly all the leaf cuttings put in at this time behaved in this manner (Pl. XX, Fig. 3, and Text-Figs. 9, 9a).

The tuber increased in size and produced roots from near the upper surface by the side of the cotyledonary petiole. Roots were not produced from the lower surface of the tuber, so that the corm was considerably different in character from the corm of a normal seedling, being smooth and rounded below and having a tuft of roots on one side only, close to the developing plumule.

The young plants developed adventitiously from the cotyledon have continued to grow and produced a plumule with several leaves and flowers (March, 1920).<sup>1</sup>

So far the regenerative capacity of the first cotyledon only has been referred to; it is of interest, therefore, to examine the behaviour of the plumular leaves under exactly similar conditions of treatment.

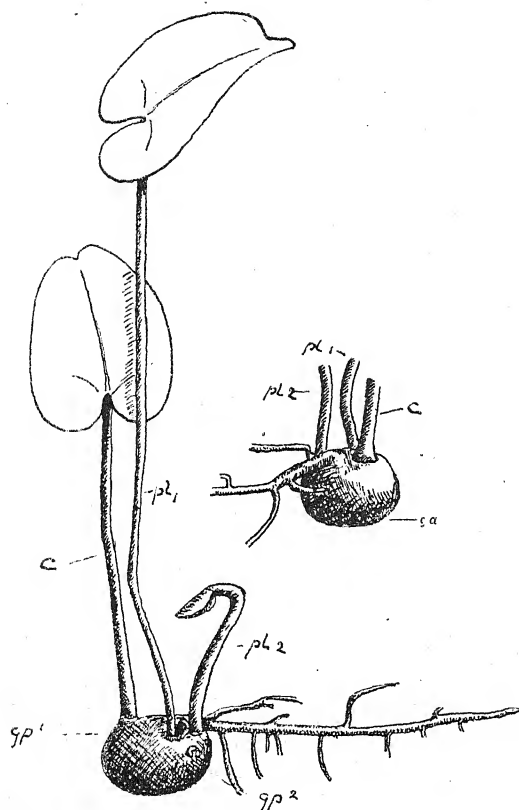
If the lamina of the first, or of any subsequent, plumular leaf be removed, no new lamina is developed from the petiole, and in the course of a few days the petiole withers and dies.

Further, such plumular leaves with their petioles were treated as 'cuttings', as described for the cotyledons, but after repeated experiments

<sup>1</sup> The behaviour of the leaves of the ivy-leaved Pelargonium when treated as cuttings was brought to the notice of the Scientific Committee of the Royal Horticultural Society, so Mr. Chittenden informs me, by Mr. Houston on October 15, 1901. As is the case with the cotyledon of *Cyclamen*, roots were freely produced from the petiole, but no buds developed. I have found, however, in the case of leaf cuttings of *Tricuspidaria lanceolata* that both roots and a shoot are produced from the base of the petiole. Ivy leaves put in as cuttings will produce leaves but no shoots, and have lived in this condition for five years, and leaves of *Hoya* in like manner for seven years (Carrière-Jardinier, Multiplicateur, s. 218). De Vries (Prings. Jahrb., xxii, 1891, pp. 68-70) refers to similar rooted leaf cuttings of *Aucuba*, *Euonymus japonicus*, *Ficus elastica*, and *Camellia japonica*, which appear to be incapable of developing adventitious buds and forming plants from leaf cuttings. He quotes from Mer (Bull. Soc. Bot. France, xxvi, p. 26), who refers to the case of the ivy-leaf cuttings.

In the Gardeners' Chronicle, Feb. 15, 1845, p. 101, D. Beaton refers to the formation of 'callosities' on the petioles of leaves treated as cuttings, and to the formation of buds in some cases; in others, as *Camellia*, the leaves lived for four years without the formation of buds.

there was no production of adventitious roots from the base of the petiole as with the cotyledon. The base of the petiole, however, enlarged considerably and formed a kind of tuber or callosity, but, though shaving of the surface of this callosity and other devices to induce root formation have been tried, the results have only been negative. The leaf cutting may remain alive for a considerable time and increase in size, but it will eventually



TEXT-FIG. 9. *C. persicum*. A first cotyledon cutting more fully developed (cf. Pl. XX, Figs. 2, 3), showing the cotyledon, *c.*, and plumular leaves, *pl. 1* and *2*. The adventitious tuber shows two growing-points, *gp 1*, *gp 2*. The root is developed from the upper surface or, more correctly, the tube is developed below the root, and is more clearly seen in the back view, *g a*.

die without having formed either root or shoot. One exception to this statement, however, must be recorded, as in one case only a young plumular leaf from a young seedling did develop a root from the base of the petiole, and the leaf cutting, which developed a strong laterally placed root, remained alive until February, 1920; there was, however, no development of an adventitious bud (Pl. XX, Fig. 1, and Text-fig. 10).

A section through the callosity or swollen base of the petiole reveals several cortical 'nests' of lignified cells—and a few such 'nests' are also

seen at the base of the cotyledonary petiole—but for some unknown reason, except in the single case mentioned, the cells of the plumular leaf appear to be unable to produce any adventitious outgrowths in the way of roots.

To return now to the second cotyledon of the *Cyclamen* seedling. It has been shown that the normal single cotyledon shows marked differences from the plumular leaves when mutilated by removal of the lamina which affords a certain means of distinguishing the two organs. It becomes therefore a matter of interest to examine the behaviour of the second cotyledon under like conditions, more especially as additional evidence may thus be obtainable in support of the contention that the embryo of *Cyclamen* is truly dicotyledonous.

From a number of seedlings in which the second cotyledon had been induced to develop, the laminae were removed, and in due course the response was exactly comparable to the traumatic reaction obtained with the first cotyledon, though, owing to the less robust character of the petiole, the percentage of successes was considerably smaller. From the apex of the petiole an outgrowth arises either on one or on both sides of the groove of its inner surface and develops to form a new assimilating organ as a flange or wing-like organ placed at right angles to the original lamina (Pl. XX, Fig. 5, and Text-figs. 11, 12).

Leaf cuttings of the second cotyledon have also been made and in the majority of cases roots have developed from the base of the petiole exactly as occurs with the first cotyledon; such rooted cuttings have not, however, produced adventitious buds or developed a plumule. As in the case of the first cotyledon there was no formation of a callosity at the base of the petiole (Text-fig. 13).

The leaves produced from the edges of the decapitated seedling tubers, being, as it may be assumed, 'cotyledonary' in character, were next tested to see whether they would respond to wounding in the same manner as do the first and second cotyledons, and it was of considerable interest to find that their response was quite similar. When put in as cuttings, roots were developed quickly from the base without a swelling being formed, but no formation of adventitious buds has so far been observed (Text-fig. 14). It may be that both with these adventitious leaves and with the second cotyledons adventitious buds might be formed if conditions should happen to be favourable.

The laminae of these adventitiously produced leaves were also removed, and a new lamina was developed from the edge of the petiole near the apex, in precisely the same manner as new laminae are produced from the petioles of the mutilated cotyledons (Text-fig. 15).

These experiments are of interest, since they tend to show that the adventitious leaves produced from the edge of the cut surface of the

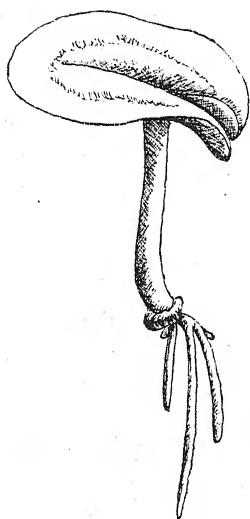


FIG. 10.

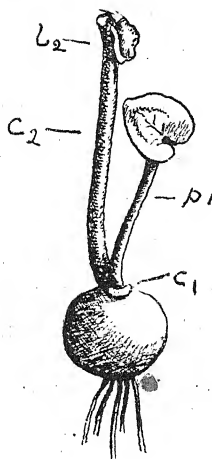


FIG. 11.



FIG. 12.

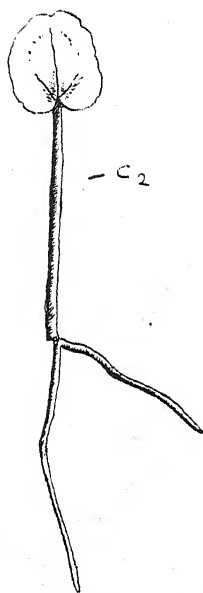


FIG. 13.



FIG. 14.



FIG. 15.

TEXT-FIGS. 10-15. *C. persicum*. 10. A first plumular leaf put in as a cutting Nov. 6, 1918, which developed a callus and also roots—the drawing was made March 21, 1919. The cutting died nearly a year later. 11. A seedling in which the second cotyledon, *c. 2*, has been produced on removal of the first cotyledon (scar, *c. 1*). The lamina has been removed, and flange-like laminae, *l. 2*, have developed from the edges of the groove on the adaxial surface of the petiole. The first plumular leaf, *p. 1*, has grown up. 12. One of the flange laminae of the second cotyledon in side view. 13. A second cotyledon struck as a 'cutting'. As with the first cotyledon, there is no swelling at the base of the petiole. 14. An adventitious leaf developed from a decapitated seedling tuber (see Pl. XX, Fig. 4, and Text-fig. 15) struck as a cutting. The roots are formed as in the cotyledon cuttings, and there is no swelling at the base of the petiole. 15. A tuber of a seedling *Cyclamen* from which the upper portion has been removed, showing one fully developed adventitious leaf and others as marginal protuberances. The lamina of the leaf was removed, and a new lamina, *l. 2*, has developed from the surface of the petiole exactly as occurs when the lamina of a cotyledon is removed.

decapitated hypocotyledonary tuber are still in the 'embryonic' condition and are comparable both physiologically and morphologically to the cotyledons.

Older tubers which had given rise to plumular leaves were also decapitated for comparison with the seedling tubers, but it was found that in such cases the adventitious leaves were produced not from the edges of the cut tubers, but from the cut surface itself close to the centre of the tuber (Pl. XX, Fig. 6). These leaves were plumular in appearance, and were found to behave as do the normally produced plumular leaves. New laminae could not be produced from the petioles when the original laminae were removed, nor could the leaves be induced to form roots when treated as 'leaf cuttings', though they formed a callus in the same manner as do the plumular leaves.

In both the decapitated seedling and adult tubers one or more plumular buds will develop in course of time and apparently normal *Cyclamen* plants result. The tubers become rounded above by the growth of the tissues of the tuber which are protected by corky tissue. They differ, however, in most of the cases examined in having several plumular shoots instead of the single axis developed by normal plants (Pl. XX, Fig. 7).

#### SUMMARY.

Arguments have been brought forward on morphological grounds to show that the rudimentary curved body lying opposite the cotyledon proper in the *Cyclamen* embryo is the second cotyledon, and that under ordinary conditions it does not develop to form a green leaf.

When artificially induced to develop, however, it is found to respond to the traumatic stimulus of the removal of the lamina by regenerating a new lamina or laminae from the petiole, exactly as does the normally developed first cotyledon.

The evidence afforded by this power of regeneration may therefore be accepted as definite proof that the embryonic rudiment is undoubtedly the undeveloped second cotyledon.

The *Cyclamen* seedling, though aberrant in type, is thus seen to be truly dicotyledonous in nature.

Thus, though the second cotyledon of *Cyclamen* has ceased to function as an absorbent organ in the seed, possibly in correlation with the geophilous habit of the genus, it is of interest to find that, when stimulated to develop as an assimilating organ, it is in the physiological state characteristic of and peculiar to the cotyledon proper—a condition which is not shared by any of the plumular leaves which arise subsequently from the shoot.

It has also been found that the leaves adventitiously developed from the edge of decapitated seedling tubers—the swollen hypocotyl—respond to traumatic stimuli in precisely the same manner as do the cotyledons.



# EXPLANATION OF PLATE XX.

Illustrating A. W. Hill's paper on Studies in Seed Germination.

The photographs have kindly been taken by Mr. J. Hutchinson, The Herbarium, Kew.

All the specimens figured in the plate are *Cyclamen persicum*.

Fig. 1. *Cyclamen*. A young plumular leaf put in as a cutting. A callus has developed, but no roots have been formed; the leaf lived in this condition for about a year.

Fig. 2. *C*. A cotyledon—the normally produced first cotyledon—put in as a cutting. Roots are quickly developed from the base, and there is no formation of a knob of callous tissue.

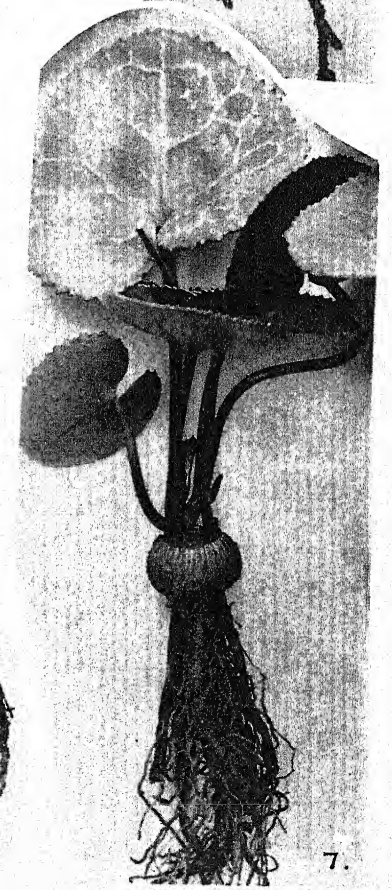
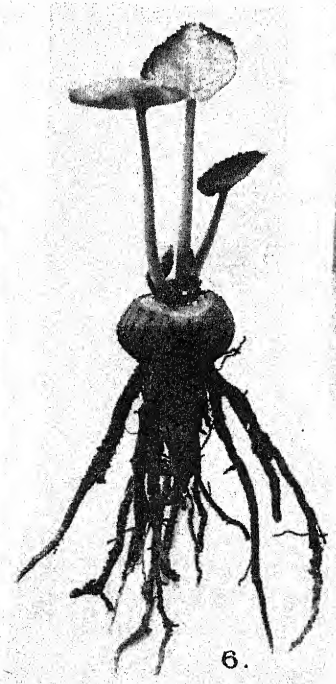
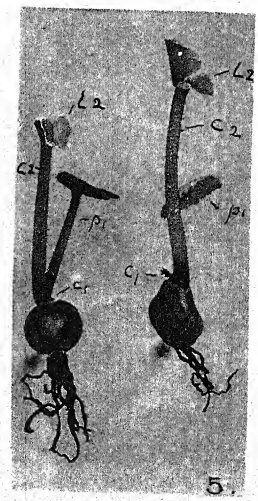
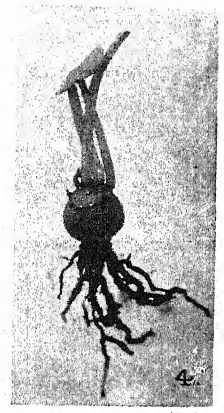
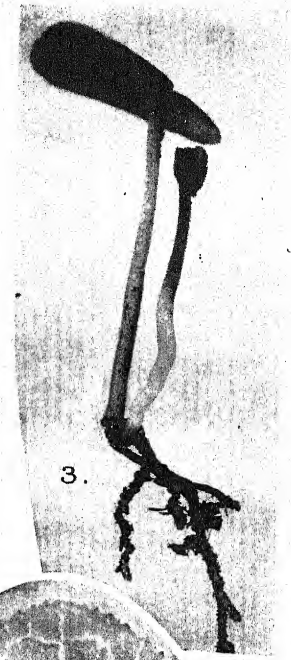
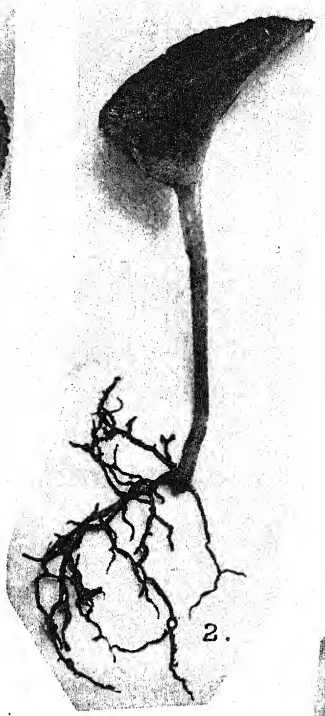
Fig. 3. *C*. A cotyledon cutting, showing a further stage of development. A tuberous swelling has grown out at the base of the root, and on this a plumular bud has formed which has given rise to plumular leaves and shoots; the lower side of the tuber is rounded and the roots are produced from the upper surface.

Fig. 4. *C*. A decapitated seedling tuber, showing the development of adventitious leaves from the surface of the tuber just below the cut surface. The adventitious leaves behave exactly as do the cotyledons.

Fig. 5. *C*. Two seedlings, from which the first cotyledon has been removed. The scar, *c. 1*, can be seen. The second cotyledon, *c. 2*, has developed, and the lamina has been removed. In each case new laminae have developed from the adaxial side of the petiole.

Fig. 6. *C*. An older tuber decapitated. In this case several plumular leaves had developed before the upper half of the tuber was removed. New adventitious plumular buds have been formed from the centre of the cut surface of the tuber, and not from just below the margin of the cut as in the seedling. The adventitious leaves in the case of these adult tubers behave as do the plumular leaves.

Fig. 7. *C*. A decapitated seedling tuber, about a year old, which has developed two plumular axes from the margin. The surface of the cut can still be distinguished.





# The Mode of Origin and the Vascular Supply of the Adventitious Leaves of *Cyclamen*.

BY

L. A. BOODLE.

With six Figures in the Text.

THE mode of origin of the adventitious leaves which arise near the margin of the cut surface of decapitated seedling-tubers or hypocotyls of *Cyclamen*<sup>1</sup> has been studied at various stages of their development, and the evolution of the vascular supply to these adventitious leaves has been traced by means of longitudinal and transverse sections.

The first stage in the formation of an adventitious leaf can be recognized by the appearance of division-walls in a small group of cells belonging to

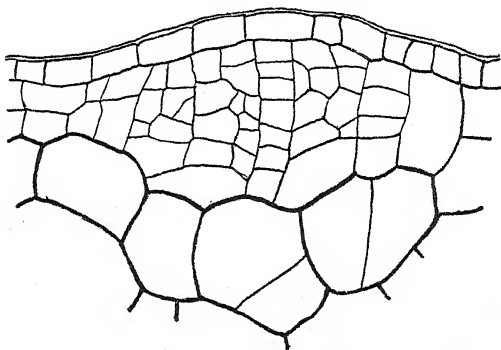


FIG. 1. Rudiment of adventitious leaf as seen in a transverse section of the tuber.  $\times 250$ .

the first layer beneath the epidermis of the young tuber. The first division-walls are tangential; and then further subdivision of the cells takes place by means of walls in different directions. Growth accompanies these divisions, a lenticular mass of small cells being produced (Fig. 1). At this stage only a minute external prominence is caused, but the latter increases in size, owing to continued growth and cell-division, and soon, e.g. when 0.2 mm. in length, bears on its surface a number of short glandular hairs, similar to those found on the young cotyledon, tuber, &c. These hairs being crowded together on the leaf-rudiment, make it easily visible.

<sup>1</sup> These leaves mostly grow from the uninjured surface of the tuber, a short distance below the margin of the cut surface.

The tissue produced by division of the sub-epidermal cells gives rise to the internal tissues of the leaf-rudiment, the epidermis of the latter being derived from that of the tuber. The portion of epidermis outside the mass of small cells belonging to the early stage of the leaf-rudiment (see Fig. 1), no doubt, stretches slightly at first, and then, as growth beneath it continues, it must undergo rejuvenescence, its cells (or a central group of them) growing and dividing so as to keep pace with the extension of the internal tissues. The original cuticle belonging to this epidermis apparently becomes exfoliated during the enlargement of the rudiment, pieces of partially detached cuticle having been observed in some cases.

The above description refers to specimens in which no periderm had been produced. Some of the tubers examined, however, were older and had begun to form periderm apparently before the origin of some of the adventitious leaves. One or two early stages of leaf-rudiments were examined, and found to lie immediately beneath the layer of cork-cells, and in lateral contact with the unsubsized layers of the periderm. Later stages showed the cork-layer ruptured, and the young leaf protruding. As the periderm arises in the sub-epidermal layer of the tuber, the epidermis of the leaf, in the cases under consideration, is consequently not derived from that of the tuber, but, together with the inner tissues of the leaf, from portions of the cells of the sub-epidermal layer, or from products of the phellogen.

Thus, taking the cases of the young and older specimens together, it appears that the internal tissues of the leaf are derived from the sub-epidermal layer of the tuber, either directly or indirectly, but that the epidermis of the leaf may have either an epidermal or sub-epidermal origin.

At the stage of the leaf-rudiment shown in Fig. 1, no procambial strand has been formed, but the appearance of a procambial connexion with the stele of the tuber is not long delayed. In Fig. 2 a rudiment of a leaf about 0.5 mm. in length is seen to be connected with the central cylinder by a procambial leaf-trace. The formation of the procambial tissue progresses from without inwards, i. e. from the leaf-rudiment towards the stele, and the progress is presumably rapid, only a few examples of traces stopping short of the vascular ring having been observed. One of the traces referred to reached the endodermis, and one died out half-way across the cortex.

The cells of the procambial trace are formed by repeated divisions in the rather large parenchymatous cells of the cortex of the tuber. A transversely cut procambial trace (perhaps not yet complete), shown in Fig. 3, is seen to have arisen by the subdivision of a single cell, at the level of the section figured. A portion of another trace cut longitudinally is represented in Fig. 4, in which the appearance is suggestive of ordinary cambial divisions.

Comparison with the preceding figure, however, makes it clear that the narrow cells of Fig. 4 are also shallow, and belong to a bundle of rod-shaped cells, traversing the original large parenchyma-cell from which they have been cut out.

The procambial tissue supplying a single leaf-rudiment becomes connected with two (Fig. 2) or sometimes with three bundles of the vascular ring, and somewhat later develops into vascular tissue. The elements of the latter are short, their length being usually about the same as the diameter of the cells from which they were originally cut out. The phloem-elements remain narrow, but the xylem consists of vessels and tracheides of greater diameter, the segments or elements being often rather broad in proportion to their length, and occasionally isodiametric.

One case was noted in which a leaf-trace had not completed its xylem connexion with the vascular ring, vessels being present only for a certain

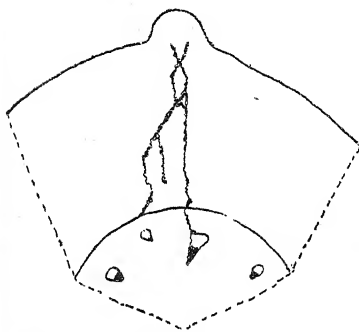


FIG. 2.

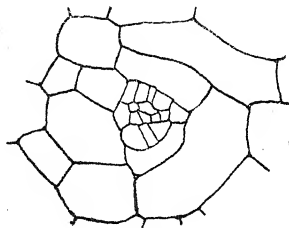


FIG. 3.

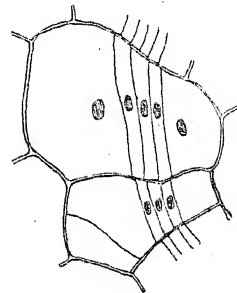


FIG. 4.

FIGS. 2-4. 2. Portion of transverse section of tuber, showing procambial trace of young adventitious leaf. Some parts of the trace are missing, being outside the thickness of the section.  $\times 20$ . 3. Transversely cut procambial strand of trace of adventitious leaf.  $\times 260$ . 4. Longitudinal section through a portion of a similar trace.  $\times 260$ .

distance from the leaf-rudiment towards the stele. The differentiation of the xylem of the leaf-trace may therefore begin at the periphery of the tuber and proceed inwards, or perhaps it may commonly begin simultaneously along the whole course of the trace, the instance noted above being an exception.

A vascular connexion between leaf and stele is shown somewhat diagrammatically in Fig. 5. Some portions of the leaf-trace are missing, though a rather thick section was used so as to include the greater part of the connexion. The leaf-trace may exhibit considerable irregularities, including division into two or more strands, and re-fusion of these, during its course, but, speaking generally, it passes inwards in relation to a radial plane and with a slightly downward inclination.

The vessels and tracheides of the leaf-trace usually show either reticulate thickening or scalariform or ordinary pitting, only a few cases in

which spiral thickening was present having been observed. The end-walls of the segments of the vessels have each a simple perforation, which is often small, and may not be recognizable in an optical longitudinal section of a vessel. The general appearance of some vessels is shown in Fig. 6, which represents a small portion of a leaf-trace. The phloem-strand (*ph.*), indicated in the figure, appears discontinuous owing to the irregularity of its course.

Transverse sections of the leaf-trace may present very different appearances at different points in its course through the cortex. In one case, for instance, the petiole has a typical arched bundle, which, on entering the tuber as the leaf-trace, shows rearrangement of its tissues, the xylem-elements first changing their course so as to be cut longitudinally in a tangential section of the tuber, thus giving the appearance of a 'plate of xylem.' By a further change of direction there is an almost immediate

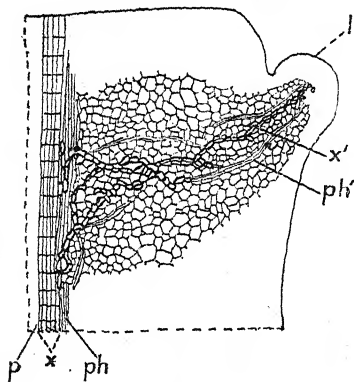


FIG. 5.

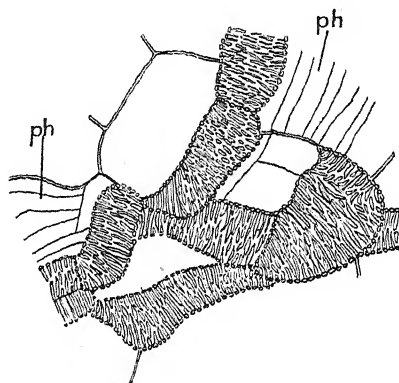


FIG. 6.

FIGS. 5, 6. 5. Longitudinal section of tuber, partly diagrammatic, showing vascular supply of adventitious leaf; *x.* and *ph.*, xylem and phloem of stele; *x'* and *ph'*, xylem and phloem of leaf-trace; *p.*, pith; *l.*, leaf.  $\times$  about 22. 6. Portion of trace of adventitious leaf cut longitudinally; *ph.*, phloem.  $\times$  260.

return to the arched form of bundle, the diameter of the arch being greater than before, and the xylem becoming somewhat interrupted. The trace then, by a slight change, assumes the form of a more or less broken ring of xylem and phloem (ectophloic), and retains this form for some distance, but with irregularities in the course of its tissues. After becoming once more arched, the trace divides into two or three strands preparatory to fusion with the central cylinder. The phloem-strands are sometimes rather widely separated from the xylem.

In one or two cases the petiolar bundle was not arched, but had a small solid cylinder of xylem surrounded by phloem, and this type of structure also was met with in portions of some leaf-traces. Small strands connecting adjoining traces sometimes occur, and, when several leaves are inserted close



together, their traces may become grouped more or less in a ring. No special morphological significance is attached to the form or course of the leaf-traces.

On examining an adventitious leaf, the lamina of which had its back towards the tuber, it was found that the petiolar bundle was still reversed at the point of attachment of the petiole to the tuber. A partial rotation of the bundle, however, took place at once on entering the tuber.

The leaves referred to so far are those produced on the uninjured surface of the tuber. Other adventitious leaves, however, had been formed on the cut surface of the tuber, but only in a small proportion of the specimens examined. Nothing specially noteworthy was observed in these leaves. Where they occurred near the central region of the cut surface, their vascular traces became attached to the bundles of the stele, not far below the cut ends of the latter, the traces having a very short and nearly vertical course.

Probably a leaf-trace can be formed in the cortex of any part of the tuber, the necessary condition in any given region being that a young leaf-rudiment should first be formed there.

No definite opinion has been obtained as to the nature of the stimulus requisite for the initiation of the leaf-trace in *Cyclamen*, but reference may be made to Simon's conclusion regarding somewhat analogous phenomena observed by him in *Achyranthes* and other plants.<sup>1</sup> In Simon's experiments a young stem was partially cut across transversely, so that some of the vascular bundles were severed, and observations were made on the development of new bundles, which were formed from parenchymatous tissue in such a manner as to connect the severed bundles above the cut either with uninjured bundles, or with severed bundles below the cut. The development of the new bundles began in parenchyma adjoining the cut ends of bundles above the incision (i. e. in tissue at first somewhat depleted of water<sup>2</sup>), and proceeded towards bundles having a direct vascular connexion with the root, and consequently an efficient water-supply. Simon concludes, from the results of his experiments, that there is a stimulus depending on the distribution of water in the tissues, and draws a comparison between the reaction (of bundle development towards the water-supply) and the phenomenon of hydrotropism.

If one supposes the stimulus in *Cyclamen* to be likewise connected in some way with local scarcity of water, one must assume a degree of depletion of water in the cells adjoining the leaf-rudiment, at an early stage of the latter. This might possibly be due to increased cuticular transpiration

<sup>1</sup> Simon: Experimentelle Untersuchungen über die Entstehung von Gefässverbindungen. Festschr. deutsch. bot. Gesellsch., 1908, p. 364. See also Küster: *Progressus Rei Botanicae*, vol. ii, pp. 541-9; and Freundlich: *Entwicklung u. Regeneration von Gefässbündeln in Blattgebilden*. *Jahrb. f. wiss. Bot.*, vol. xlv, 1909, p. 137.

<sup>2</sup> Owing to interruption of vascular supply of water from below.

of the rejuvenated portion of the epidermis, combined with the transference of water, required for the enlargement of the rudiment and for the production of hairs on its surface.

Goebel,<sup>1</sup> referring to the production of these adventitious leaves in *Cyclamen*, states that, as far as is known, they arise directly on the hypocotyledonary tuber, and this certainly appears to be the case, nothing suggesting the preliminary differentiation of an adventitious stem-apex having been observed. Goebel, moreover, regards the production of adventitious leaves as indicating the 'embryonic' nature of the hypocotyledonary region of the seedling.

Certain exogenous buds and roots may be compared with the adventitious leaves of *Cyclamen*, in that they also require the formation of a vascular connexion across the cortex. In *Aristolochia Clematidis* adventitious buds are formed on the roots, and according to Beijerinck's description<sup>2</sup> they show some interesting analogies to the adventitious leaves of *Cyclamen*. Thus the differentiation of the bud-trace proceeds centripetally through the cortex of the root, and forking of the trace occurs (Beijerinck, Fig. 82). Further, the epidermis of the bud may vary in origin according to the early or late development of the bud, being derived from the outermost layer of the root, or from the next layer beneath.<sup>3</sup>

Beijerinck adds that the chief part of the bud arises from the two or three layers of cells which immediately adjoin the 'epidermis', and may be regarded as cork-meristem.

The formation of exogenous adventitious buds on the hypocotyl has been recorded in species of *Linaria*, *Anagallis*, *Euphorbia*, and other genera.<sup>4</sup> In these cases the hypocotyl is of normal form, not tuberous as in *Cyclamen*, and the production of the buds may be spontaneous, but is favoured or increased by the removal of the cotyledons and stem-apex of the seedling, or by encasing these in gypsum, or by the accident of the cotyledons remaining enclosed in the seed-coat.<sup>5</sup> As adventitious buds do not appear to occur on the epicotyl of these plants, a pronounced formative property may be taken as here characterizing the hypocotyl. The production of adventitious leaves by the tuber of *Cyclamen* may be classed with the foregoing cases as another example of an active hypocotyl, and

<sup>1</sup> Goebel: *Einleitung in die Experimentelle Morphologie*, 1908, p. 205.

<sup>2</sup> Beijerinck: *Beobachtungen u. Betrachtungen über Wurzelknospen u. Nebenwurzeln*. *Natuurk. Verh. Akad. Wetensch. Amsterdam*, vol. xxv, p. 107.

<sup>3</sup> Beijerinck, loc. cit.: 'Die dunkelschwarze, Rinde und Knospe überziehende Epidermis wird gewöhnlich durch die Knospe durchbohrt, allein bei sehr früh angelegten Knospen ist die Epidermis der Mutterrinde ein integrierender Theil der Neubildung.'

<sup>4</sup> Goebel: loc. cit.

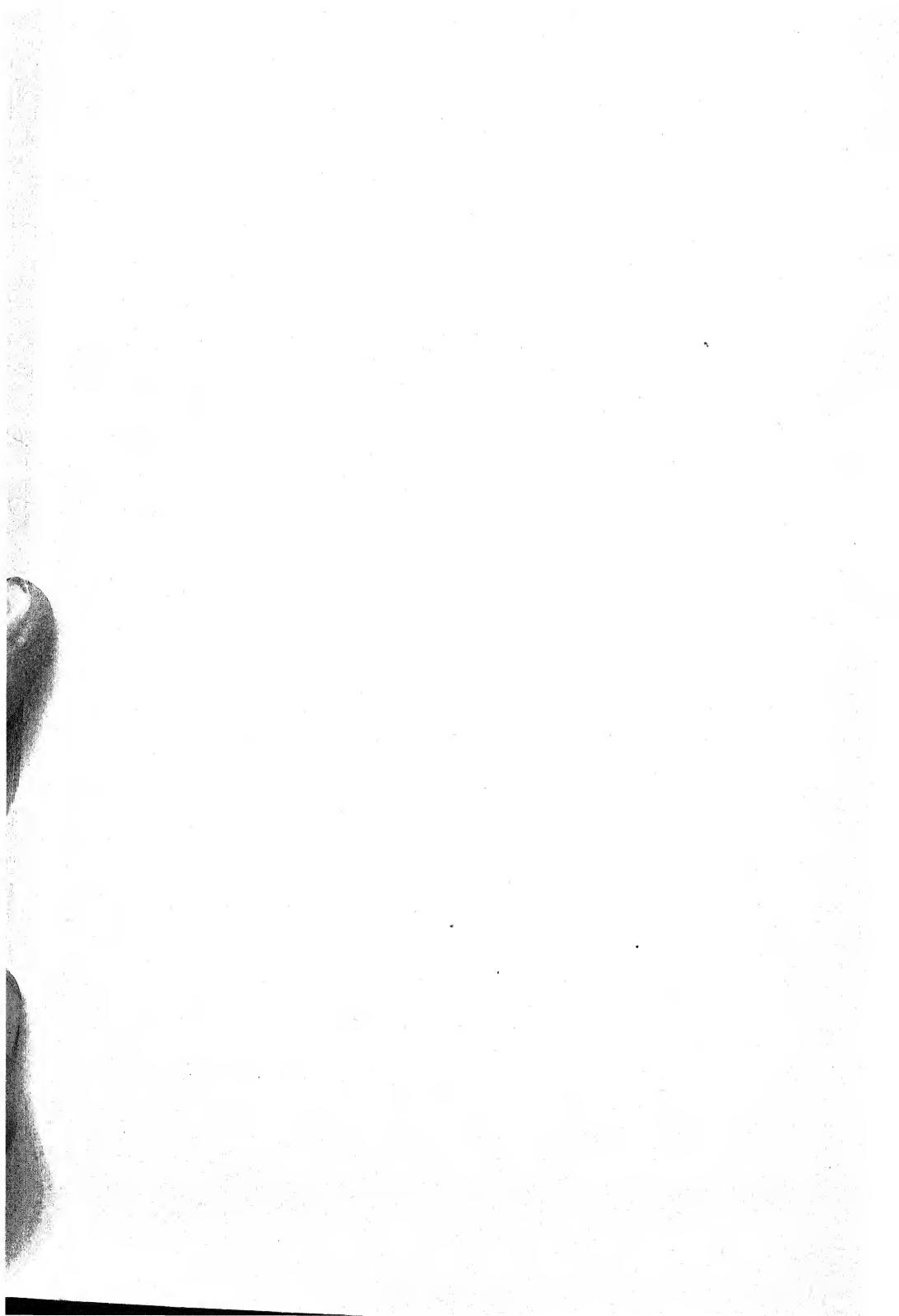
<sup>5</sup> Küster: *Beobachtungen über Regenerationserscheinungen an Pflanzen*. *Beih. z. Bot. Centralbl.*, vol. xiv, 1903, p. 316. Burns and Hedden: *Conditions influencing Regeneration of Hypocotyl*. *Ibid.*, vol. xix, 1906, p. 381.

the phenomenon in *Cyclamen* should probably not be regarded as primarily connected with the tuberous nature of the hypocotyl.

Reference may also be made to the exogenous roots formed in connexion with the axillary buds on the stem of *Nasturtium officinale*.<sup>1</sup> Here again vascular strands are developed from cortical tissue, but the tissue concerned is not mature as in *Cyclamen*, the mode of differentiation of the vascular elements being comparable to that occurring in the development of normal primary vascular tissue.

Among the results obtained, the most interesting are those connected with the origin of the adventitious leaves. The latter, to summarize, may be produced: (1) strictly exogenously, when there is no periderm, or (2) just below the cork, when periderm has been formed, or (3) beneath the cut surface, even from cortical cells quite near the stele. In the first case the epidermis of the leaf is derived from that of the tuber, while in the second it is subepidermal in origin.

<sup>1</sup> Lemaire: Recherches sur l'origine et le développement des racines latérales. Ann. Sci. Nat., Bot., 7<sup>e</sup> sér., t. iii, p. 237.



# The Rôle of the Seed-coat in Relation to the Germination of Immature Seed.

BY

FRANKLIN KIDD

AND

CYRIL WEST.

With six Tables and one Chart in the Text.

IN previous papers (9 and 10) it has been shown that the germination of seeds of *Brassica alba*, sown in the presence of certain percentages of carbon dioxide, can be completely inhibited, and that this inhibition of germination is often maintained indefinitely after the removal of the seeds to air. This is a remarkable phenomenon, and it is the more striking in that seeds rendered dormant by carbon dioxide show no signs of injury when finally brought to germination even after the lapse of twelve months. So far the authors have found only two certain methods of destroying the dormant condition into which the seeds are thrown by the carbon dioxide treatment. One method is to redry the seed, the other method is to remove the testa without drying.

In the present paper results are recorded which show that a condition of dormancy, similar in many ways to that produced when dry mature seeds of *Brassica alba* are sown in the presence of carbon dioxide, may be observed when immature seeds of this plant are sown immediately after removal from the parent and before the natural drying process has begun.

## EXPERIMENTS WITH *BRASSICA ALBA*.

The seeds in the following experiments were obtained from plants grown in the Botanic Gardens, Cambridge. The different degrees of ripeness of the seeds were as follows:

- A. *Green-ripe*: Seeds fully swollen; still quite green; 50 per cent. to 80 per cent. of the dry weight of ripe seeds. Siliques bright green.
- B. *Yellow-ripe*: Seeds fully swollen; yellow in colour; of practically the same dry weight as fully ripe seeds. Siliques still moist; beginning to turn yellow.

The germination results are set forth in the following tables (Tables I-IV) and chart. Yellow-ripe seeds with the testa intact, when sown

immediately after removal from the parent, remained in a dormant condition or showed a long delay in germination. On the other hand, yellow-ripe seeds with the testa removed, or dried in the laboratory for twenty-four hours with the testa intact, germinated immediately (100 per cent.), even more rapidly than a sample of the previous year's seed used as a control.

Green-ripe seeds sown after the removal of the testa germinated completely (100 per cent.), but after a delay of a few days. When sown with the testa on they mostly died, and in the case of the few seeds which did germinate, it was noticed that germination was always preceded by a change in the colour of the radicle from green to yellow. If dried for forty-eight hours before sowing practically all the seeds were killed.

TABLE I.

*Germination of White Mustard Seeds at different stages of maturity.*

- Lot A. Twenty yellow-ripe seeds gathered and removed from the siliques on Sept. 8, 1918. Dried in the laboratory for one day.  
 Lot B. Twenty yellow-ripe seeds gathered on Sept. 8 and removed from the siliques on Sept. 9. Sown immediately (i. e. not dried).  
 Lot C. Twenty green seeds gathered and removed from the siliques on Sept. 8. Sown immediately (i. e. not dried).  
 Lot D. Twenty green seeds gathered and removed from the siliques on Sept. 8. Testas removed before sowing.

All the seeds were sown on moist silica sand in a glass thermostat at 20° C.

	Germinations after:									
	16 hours.	40 hours.	45 hours.	65 hours.	4 days.	5 days.	7 days.	9 days.	12 days.	21 days.
Lot A	1	16	18	20	—	—	—	—	—	—
" B	0	0	4	9	10	13	13	14	15	20
" C	0	0	0	0	0	1	5	8	10	14*
" D	0	0	0	0	2	3	20	—	—	—

\* The 6 ungerminated seeds were dead.

TABLE II.

*Germination of White Mustard Seeds at different stages of maturity.*

All the seeds were sown on moist silica sand in a thermostat at 20° C.

Kind of Seed sown (sown on Sept. 11).	Number of Seeds sown.	Germinations after:							
		18 hours.	24 hours.	41 hours.	65 hours.	5 days.	7 days.	10 days.	19 days.
Green-ripe, testas off	10	0	0	0	0	7	10	—	—
" " on	10	0	0	0	0	0	0	—	—
Yellow-ripe, testas off	10	9	10	—	—	—	—	1	1*
" " on	10	0	0	3	—	—	—	—	—
Dry mature seed (1917)	10	0	6	10	5	5	6	6	7†
Yellow-ripe, dried in the laboratory air for 3 days	10	3	10	—	—	—	—	—	—

\* The nine ungerminated seeds were dead.

† The three ungerminated seeds were not dead.

TABLE III.

*Germination of White Mustard Seeds at different stages of maturity.*

All the seeds were sown on moist sand in a thermostat at 20° C.

Kind of Seed sown (sown on Sept. 11).	Number of Seeds sown.	Germinations after :				
		1 day.	3 days.	5 days.	8 days.	17 days.
Yellow-ripe, testas off, dried in the laboratory air for 2 days	10	8	10	—	—	—
Green-ripe, testas off, dried in the laboratory air for 2 days	10	0	1	2	2	2*
Green-ripe, testas on, dried in the laboratory air for 2 days	10	0	0	0	0	0*

\* The ungerminated seeds were dead.

TABLE IV.

*Germination of White Mustard Seeds at different stages of maturity.*

Lot A consisted of ten green-ripe seeds.

Lot B consisted of ten bare embryos from similar seeds.

Sown on moist sand, July 14.

	2 days.	4 days.	6 days.	7 days.	12 days.	18 days.
Lot A	0	0	0	0	1	2*
„ B	0	0	5	10†	—	—

\* The 8 ungerminated seeds finally died.

† Healthy plants.

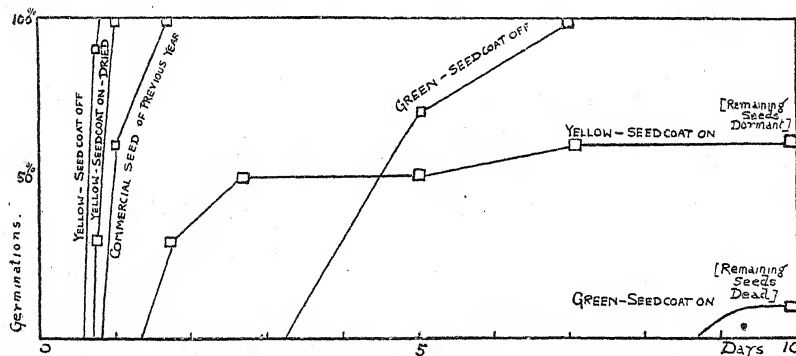


CHART. Germination of White Mustard seeds at different stages of maturity as affected by the seed-coat and by drying the seed (see Table II).

From the above experiments on the germination of immature White Mustard seeds it appears that the presence of the testa is fatal so long as the seeds are still green and have not reached their full dry weight. If they are sown without the testa they germinate in a healthy manner, but if sown with the testa on they perish. As the seed ripens, however, and



turns yellow, the effect of the presence of the seed-coats is markedly reduced. The presence of the seed-coats in the yellow-ripe stage only causes dormancy or delayed germination. Finally, when the testa is completely yellow and the seed is dry, the testa no longer has any appreciable effect upon germination.

Since the embryos will germinate freely at all three stages if the seed-coats are removed, there does not seem to be any ground for seeking an explanation of the above differences in behaviour between green-ripe, yellow-ripe, and dry-ripe seeds in progressive changes occurring in the embryo itself.

The progressive changes which occur in the testa, to which we must therefore look for an explanation, may be described as follows. In the green-ripe seed the embryo is enclosed in a relatively thick green coat consisting of actively functioning tissue, while between this coat and the embryo a considerable quantity of liquid is usually found. In the yellow-ripe seed the testa is relatively thinner and presumably less active, but is still living. The liquid between the embryo and the seed-coats has by this time disappeared. In the dry-ripe seed the testa is a thin membrane and presumably dead.<sup>1</sup>

Considered as an obstacle to the gaseous exchange of the embryo the testa probably behaves in relation to its thickness in the same way as a film of water; in other words, the thicker the testa the more slowly do gases pass through.

It is a well-known fact that immersion in water does inhibit the germination of most seeds, and that prolonged immersion is fatal (see Kidd and West, 11 and 13<sup>2</sup>).

Further, the living testa, i. e. the testa in the green-ripe and yellow-ripe stages as opposed to the dry-ripe stage, will not only consume oxygen and, as it were, steal it on its way to the embryo, but on account of its production of carbon dioxide will also tend to hinder the escape of this gas from the tissues of the embryo. The progressive changes in the testa described above will therefore for two reasons be in favour of a progressively greater concentration of oxygen available for the embryo, and a more rapid escape of carbon dioxide.

In this connexion it is interesting to note that Demoussy (5) found that hydrogen peroxide increased the percentage of germination of old Cress seeds. Hydrogen peroxide has a lethal action upon the saprophytic flora of the dead seed-coats. He suggested that non-germination in these old seeds was to some extent due to the respiratory action of moulds or bacteria present in

<sup>1</sup> For details as to the histological and microchemical characters of the testa in the case of the genus *Brassica*, the reader is referred to papers by Schroeder (21), Sempelowski (22), Holfert (8), Burchard (3), Gram (7), Kinzel (14), Pieters and Charles (20), Kondo (16), and Kidd and West (10).

<sup>2</sup> In this paper the literature dealing with the effect of soaking seeds in water is critically reviewed.

the seed-coat, attributing to them a rôle in causing dormancy similar to that which we are here attributing to the respiratory activity of the living testa.

# EXPERIMENTS WITH *PISUM SATIVUM*.

The results of certain experiments with immature seeds of *Pisum sativum* are recorded in Table V, and appear to be essentially similar to those obtained with *Brassica alba*.

TABLE V.

*Germination of seeds and bare embryos of Pisum sativum at different stages of maturity.*

- A. { Lot I. Twenty immature seeds. These had attained their full size  
(the funicle comes away with the pea when taken from the pod).  
Lot II. Twenty bare embryos from similar seeds.
  - B. { Lot I. Twenty less immature seeds (peas break from the funicle when  
taken from the pod).  
Lot II. Twenty bare embryos from similar seeds.
  - C. { Lot I. Twenty seeds picked ten days later.  
Lot II. Twenty bare embryos from similar seeds.
  - D. Twenty seeds picked at a still later stage.
  - E. Controls = Twenty mature seeds gathered during previous season.
- Seeds sown in garden soil. Temperature 15°-20° C.

		Number of Germinations after:		
		5 days.	10 days.	14 days.
A	Lot I	0	1 (19 dead)	—
	" II	20	20 (18 healthy)	—
B	" I	0	0 (all dead)	—
	" II	20	20 (all healthy)	—
C	" I	—	10 (10 ungerminated dead)	—
	" II	—	20 (all healthy)	—
D		—	—	18
E	[Controls]	20	—	—

If the testa is not removed a large proportion of the seeds perish when sown. The most immature seeds tested showed a high mortality, but as maturity was approached so the injurious effect of the testa was decreased, nevertheless the seeds that survived showed an appreciable delay in germination. No real dormancy similar to that described above in the case of *Brassica alba* was observed.<sup>1</sup>

In order to test our hypothesis that the effect of the testa, as shown in the experiments above, is to be attributed to its property in limiting the gaseous exchange of the embryo, further experiments were carried out in

<sup>1</sup> It is interesting to note here that this is the same relation between seeds of *Brassica alba* and those of *Pisum sativum* in regard to dormancy as that found when the germination of these seeds was inhibited by atmospheres containing certain percentages of carbon dioxide (cf. Kidd, 9). Seeds of White Mustard exhibit secondary dormancy when removed to air. No such phenomenon can be obtained with peas.

the following way. It was argued that, assuming the hypothesis to be correct, these immature seeds, when sown under germinating conditions, must either be in a condition in which the limitation of the gaseous exchange of the embryo is so great as to become actually harmful (e.g. in the cases where sowing on damp sand is followed by death), or at any rate must be near the point at which any further limitation will cause injury. It follows that if we impose even for a short period a condition which further limits the gaseous exchange of the embryo we should, if our hypothesis is correct, obtain a pronounced result. In order to further limit the gaseous exchange, the seeds were immersed in water for short periods before sowing. The result, as Table VI shows, is striking and bears out our hypothesis. Whereas fully ripe dry pea seeds will endure immersion in water for several hours without showing any obvious decrease in the percentage of germination (Kidd and West, 11), the unripe pea seeds suffer heavily even after a few hours' immersion. The amount of injury shown is more or less proportional to the period of soaking. On the other hand, soaking *per se* for the periods used in this experiment is in no way harmful to the embryo, as is shown by the results of a parallel series of experiments with the bare embryos from unripe pea seeds.

TABLE VI.

The seeds used in this experiment were similar to those of category C in Table V. Twelve seeds were used in each experiment. They were sown in garden soil. Temperature 15°–20° C.

Condition of the Seed.	Treatment.	Results observed 11 days after sowing:		
		Percentage of seeds dead.	Percentage of vigorous plants.	Average length of the shoots.
Immature. With testas	Dried in air for 15 hours before sowing	20	58	2 cm.
	Sown immediately after removal from parent plant	33	50	2 "
	Soaked in tap-water for 1½ hours before sowing	50	42	3 "
	Soaked in tap-water for 5 hours before sowing	58	33	2 "
Immature. Without testas	Dried in air for 15 hours before sowing	0	50	2.5 "
	Sown immediately after removal from parent plant	0	92	3 "
	Soaked in tap-water for 1½ hours before sowing	0	100	4.5 "
	Soaked in tap-water for 5 hours before sowing	0	100	4.5 "
Mature intact seeds	Soaked for 24 hours	0	100	8.5 "

The above table also shows results which were obtained with bare embryos and with intact seeds which were dried in the air for fifteen hours before sowing. The germination of the bare embryos is reduced from 100 per cent. to 50 per cent. by the drying process, which is thus shown to be injurious to unripe pea seeds. A similar reduction in the percentage of

germination occurs when the testa is allowed to remain *in situ* whether the seeds are dried previous to sowing or not. When the seeds are not subjected to the drying process before sowing, the living testa is responsible for the 50 per cent. mortality observed. When the seeds are sown after having been dried the testa is presumably a dead membrane, and it is now the drying of the seed which causes the 50 per cent. mortality observed, just as in the case of the bare embryos which had been allowed to dry.

Experiments conducted by Dr. F. F. Blackman and Miss N. Darwin (4), of which an abbreviated account was read at the British Association Meeting held at Sheffield in 1910, but of which no published record is at present available, are significant in relation to the hypothesis put forward above to the effect that the living testa of unripe seeds limits the gaseous exchange of the embryo in the same way as a continuous film of water. They worked with barley grains, the vitality of which had been reduced (by age or by immersion in the swollen condition in hot water at 50° C. (*circa*) for twenty minutes), but which still showed a full percentage of germination when sown under ideal conditions. It was found that slight films of water greatly delayed the germination, reduced the germination percentage, and resulted in the death of a large proportion of such seeds. The results obtained were more or less proportional to the thickness of the water films.

#### CONCLUSIONS.

Many authors (see especially Nobbe (19), Mazé (18), Windisch (23), Eberhart (6), Atterberg (1), Babcock (2), Kinzel (15), and Kondo (17)) have described experiments dealing with the dormancy or delayed germination observed when certain seeds, which, although immature and with a relatively high moisture content, have nevertheless attained their full size, are sown immediately after removal from the parent plant. The process of drying has generally been found to terminate the dormant condition of such seeds. Different theories have been put forward to account for the dormancy of unripe seeds sown in the moist condition immediately after removal from the parent plant (see Kidd and West, 12). In the present paper it has been shown that in the case of *Brassica alba* and *Pisum sativum* the removal of the testa not only accelerated the germination and terminated the dormant condition of unripe seeds, but also increased the germination percentage. It is clear that the rest period observed when attempts are made to germinate unripe seeds fresh from the parent plant may be largely attributed to the presence of the testa, and there are strong indications that under these conditions the *living* testa limits the gaseous exchange of the embryo. A fact which should always be borne in mind in this connexion is that the testa, considered as a membrane through which the gaseous exchange of the embryo must occur, undergoes great modifications during the ripening and drying off of the seed.

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# On the Leaf Structure of certain Liliaceae, considered in Relation to the Phyllode Theory.

BY

AGNES ARBER, D.Sc., F.L.S.,

*Keddey Fletcher-Warr Student of the University of London.*

With thirty-eight Figures in the Text.

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## INTRODUCTION.

IN a memoir<sup>1</sup> published in the 'Annals of Botany' in 1918, I traced the general results which seemed to me to follow when the Phyllode Theory was applied to the interpretation of the Monocotyledonous leaf. The present paper forms one of a subsequent series<sup>2</sup> in which I am attempting to deal in further detail with the evidence concerned, and also to follow out various lines of thought—in part already indicated in my 1918 paper—which arise when the leaf is considered from this standpoint. In this instalment I propose to discuss certain selected cases among the Liliaceae.

### I. THE LEAF-BASE PHYLLODES OF *ANEMARRHENA* (ASPHODELOIDEAE).

In a recent paper in the 'Botanical Gazette'<sup>3</sup> I have interpreted certain leaves among the Liliaceae, such as those of *Hemerocallis* and *Scilla*, as reduced to leaf-bases alone. I have pointed out that there is some evidence for this view in the fact that the petiole—though here entirely lost—may, in the case of the closely similar leaves of *Hyacinthus* and

<sup>1</sup> Arber, A. (1918).

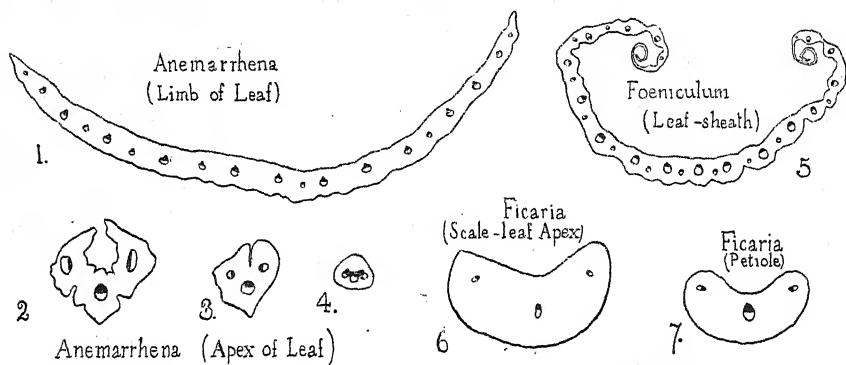
<sup>2</sup> Ibid. (1919, 1920<sup>1</sup>, 1920<sup>2</sup>).

<sup>3</sup> Ibid. (1920<sup>1</sup>).

*Tulipa*, be recognized in a vestigial condition, forming a short cylindrical apex, in which transverse sections reveal a ring of bundles.

I propose here to consider the additional case of *Anemarrhena*, since this monotypic genus seems to me to afford some slight confirmatory evidence for the existence of leaf-base phyllodes.

I chose the leaf of *Anemarrhena asphodeloides*, Bunge (Asphodeleae-Anthericineae), for examination, because, in this plant, Miss Ethel Sargent<sup>1</sup> found a type of seedling structure which a comparative study of the Liliaceae showed to be primitive for that Family. The leaf of *A. asphodeloides* is long, linear, and parallel-veined, ending in an attenuated point. It does not terminate in a relatively massive cylindrical apex with a ring of



FIGS. 1-7. (Xylem, black; phloem, white.) Figs. 1-4, *Anemarrhena asphodeloides*, Bunge. Fig. 1, transverse section of limb of leaf ( $\times 14$ ). Figs. 2-4, series of transverse sections through apical region of leaf ( $\times 23$ ) (these sections are from herbarium material, and the exact arrangement of the fused bundles in Fig. 4 could not be ascertained). Fig. 5, *Foeniculum vulgare*, Mill. Transverse section of leaf-sheath ( $\times 14$ ). Figs. 6 and 7, *Ranunculus Ficaria*, L. Fig. 6, transverse section of apex of scale leaf ( $\times 23$ ). Fig. 7, transverse section of a rather small petiole ( $\times 14$ ).

bundles, such as I have described for *Hyacinthus*, &c. But a series of sections through the apical region shows that, as the leaf narrows down, it becomes deeply grooved on the upper side (Fig. 2), and the vascular system is reduced to three veins, of which the two laterals come to lie almost horizontally. The groove gradually disappears (Fig. 3), while the bundles fuse into a single vascular mass (Fig. 4). The apical structure of this leaf seems to me to be readily interpreted on the view that the entire leaf is of 'leaf-base' nature, and that the slender apex represents the region which, in the ancestral leaf, formed the transition to the petiole. The relation of the limb to the apex closely recalls the relation of these parts in the scale-leaf of *Ranunculus Ficaria*, L. (Arber, A., 1918, *pr.* in Fig. 4, p. 474), which is undoubtedly of leaf-base nature. Sections through the apex of this scale (Fig. 6) show three bundles occupying the same relative position as the three bundles of the *Anemarrhena* leaf-tip, and this structure is also

<sup>1</sup> Sargent, E. (1903).



characteristic of the petiole in the case of the foliage leaf of *R. Ficaria* (Fig. 7).

The limb of the leaf of *Anemarrhena* is characterized by a single series of normally orientated bundles (Fig. 1), among which, however, the midrib is not well defined. This, again, is distinctly a leaf-base character. A similar lack of *obvious* symmetry about a midrib is found, for instance, in the sheathing leaf-base of the Umbellifer, *Foeniculum vulgare*, Mill. (Fig. 5).

That some leaves among the Monocotyledons should be reduced to leaf-bases alone, ceases to be surprising when we remember how strongly developed this region is apt to be in the leaves of this Class as compared with Dicotyledons. The existence of a tendency towards the preponderance of the leaf-base is suggested not only by the countless Monocotyledons which have conspicuously long leaf-sheaths (e.g. many Gramineae, and species of *Allium*, *Veratrum*, &c.), but also by the numerous bulbs in which this region, largely developed and utilized for food storage, survives the death of the remainder of the leaf. Among Dicotyledons with well-marked leaf-sheaths, we can trace the actual process of reduction from normal leaves to scale leaves consisting of leaf-bases alone. The Umbelliferae furnish obvious examples—examples that were, indeed, known to the ancients. One of the most famous manuscripts of Dioscorides—the Vienna Codex associated with the name of Juliana Anicia, which dates back to the sixth century A.D.—includes a beautiful drawing of an Umbellifer called 'Sphondylion', in which every gradation is represented between normal foliage leaves and leaves of a definitely Monocotyledonous facies, in which the leaf-base alone is developed.

The leaf-base phyllodes among the Monocotyledons may be regarded as representing the ultimate term in that arrest of apical growth which Professor Bower,<sup>1</sup> in a recent memoir on 'leaf-architecture', has recognized as a significant factor in foliar evolution. He points out that this arrest may go so far that 'the effective region originates basally'. In extreme cases—such as the protective scales of certain Osmundaceae, Cycadaceae, and Angiosperms—it may even 'involve the atrophy of the whole distal region'.

## II. THE PETIOLAR PHYLLODES OF *ASPHODELUS* AND *EREMURUS* (ASPHODELOIDEAE).

The genera *Asphodelus* and *Eremurus* were briefly cited in my 1918 paper as examples of phyllodic anatomy from among the Asphodeloideae-Asphodelineae; I propose here to describe the leaf structure in these cases

<sup>1</sup> Bower, F. O. (1916). I regret that I did not know of this memoir in time to cite it in my general paper on the 'Phyllode Theory' (Arber, A., 1918); though dealing primarily with the Ferns, it also includes a very suggestive discussion, on broad lines, of the leaf morphology of the higher plants.

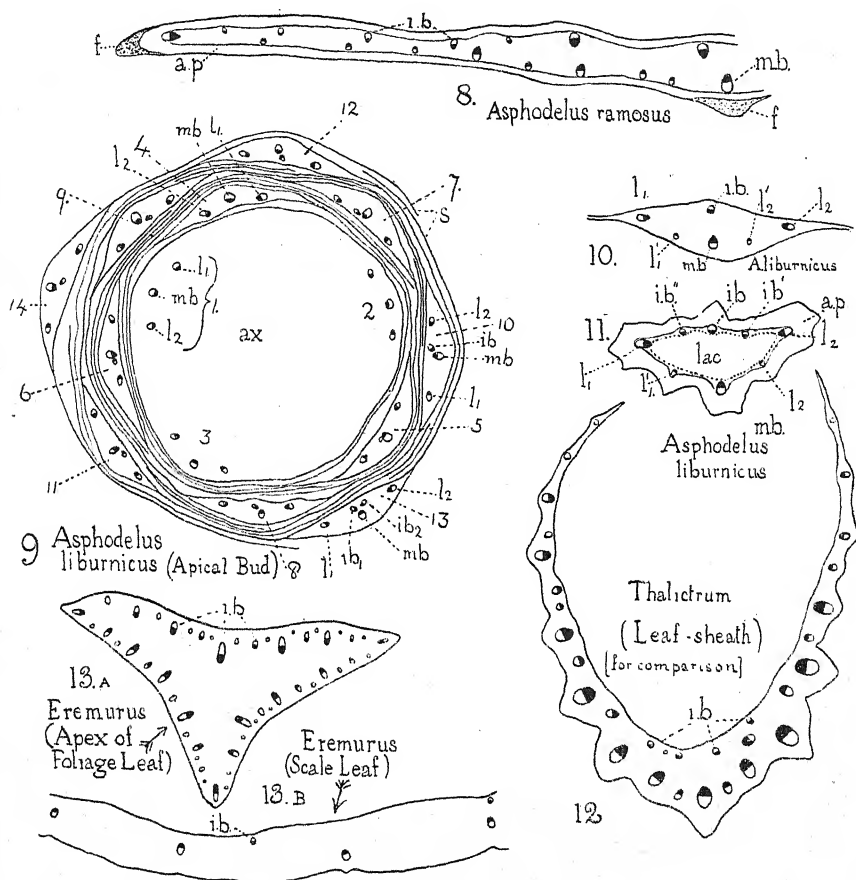
in some little detail. *Asphodelus liburnicus*, Scop. (*Asphodeline liburnica*, Reichb.), may be taken as an example of those species of *Asphodel* which have a more or less centric type of leaf; the mature limb is roughly triangular, but with an extra ridge in the median line of the adaxial (upper) surface—the base of the triangle—and subsidiary ridges between the four main angles (Fig. 11). The leaf structure is best understood from the consideration of serial sections through an apical bud, such as that represented in Fig. 9. The section is taken below the level of attachment of leaves 1, 2, and 3, and their vascular supply is still included within the axis. In the case of each leaf, there is, from the beginning, a median bundle, *m.b.*, and a lateral on either side, *l*<sub>1</sub> and *l*<sub>2</sub>. Leaves 4 and 5 are free from the axis, but their membranous wings form a closed sheath round it. In the succeeding leaves the sheath, though still a conspicuous feature, is open; the closed region is thus extremely short. In leaves 5 and 6 the midrib bundle is in the act of branching, and in leaf 7, and all successive leaves, the vascular strand, *i.b.*, which it gives off, is entirely free. As is shown in Fig. 9, the bundle, *i.b.*, is sometimes derived from one side of the median strand and sometimes from the other, in a way that seems to be quite fortuitous; I have not been able to discover that there is any regularity or rhythm in the right-handed or left-handed origin of this strand in successive leaves. But, whether it be given off to one or other side, the bundle in question gradually moves round and eventually places itself opposite to the median bundle, towards the xylem of which its xylem is turned. We meet with a similar case in the median bundle of the leaf of *Tritonia* (Iridaceae), which also gives off a lateral branch which immediately takes up an inverted position, but here the parent bundle and its branch remain in close association and form a double bundle (Arber, A. (1918), Fig. 15, p. 483).<sup>1</sup> In *Asphodelus liburnicus* each of the lateral bundles (*l*<sub>1</sub>, *l*<sub>2</sub>) gives off a branch (*l*<sub>1</sub>' and *l*<sub>2</sub>') which lies towards the lower surface of the leaf between the midrib and the lateral angles (Fig. 10). The adaxial bundle, *i.b.*, gives rise in many cases to two branches (*i.b.*' and *i.b.*''), so that there are three inverted bundles towards the upper surface of the leaf (Fig. 11). An example of an anomaly, which occasionally occurs, is seen in leaf 13, Fig. 9. Here the median bundle gives off two inverted bundles, *i.b.*<sub>1</sub> and *i.b.*<sub>2</sub>, instead of the single bundle, *i.b.*

It will be observed that in *A. liburnicus* the region which is anatomically of leaf-base nature is very short, as the inverted adaxial bundle quickly comes into being, thus rendering the vascular symmetry rather petiolar than 'leaf-base' in character. This reduction of the leaf-base is, as we shall see, carried still farther in *Eremurus*.

The leaves of plants belonging to the genus *Asphodelus* are not all

<sup>1</sup> Chodat, R., and Balicka-Iwanowska, G. (1892). The present writer has confirmed these authors' description of the origin of the double bundle.

centric in form like *A. liburnicus*. *A. ramosus*, L., for instance, has a flat linear leaf which—at least as far as can be judged from herbarium material



FIGS. 8-13. (Xylem, black; phloem, white; fibres, dotted.) Fig. 8, *Asphodelus ramosus*, L. Transverse section of half a leaf, including median bundle (m.b.); f. = fibres; a.p. = assimilating parenchyma; i.b. = inverted bundle. (This section was from herbarium material, which possibly had not recovered its normal thickness) ( $\times 14$ ). Figs. 9-11, *Asphodelus liburnicus*, Scop. Fig. 9, transverse section near apex of axis, ax., showing a number of young leaves (1-14) with divergence  $\frac{1}{2}$  ( $\times 14$ ). In each leaf, m.b. = median bundle; l<sub>1</sub> and l<sub>2</sub> = lateral bundles; i.b. = inverted bundle derived from median bundle. In leaf 13, two bundles, i.b.<sub>1</sub> and i.b.<sub>2</sub>, are derived from the median bundle. s. = sheathing wings of leaf-base. Fig. 10, transverse section through another leaf cut at a higher level, showing l<sub>1</sub>' and l<sub>2</sub>', which have been given off from l<sub>1</sub> and l<sub>2</sub> ( $\times 14$ ). Fig. 11, transverse section, higher still in the limb of another leaf, showing i.b.' and i.b.', which have been given off from i.b.; lac. = lacuna; a.p. = assimilating parenchyma. Fig. 12, *Thalictrum flavum*, L. Transverse section of leaf-sheath to show inverted bundles, i.b. ( $\times 14$ ). Fig. 13, *Eremurus himalaicus*, Baker. Fig. 13 A, transverse section near apex of foliage leaf ( $\times 14$ ). Fig. 13 B, part of transverse section of scale leaf ( $\times 14$ ); i.b. = inverted bundle.

—would scarcely, from its external appearance, be suspected of phyllodic characters. But sections reveal two rows of bundles—the upper ones inverted—and a horizontally placed marginal strand (Fig. 8); the whole

structure distinctly recalls the horizontally expanded phyllode of *Acacia leptospermoides*, Benth. (Fig. 27, p. 457).

The leaf of *Eremurus himalaicus*, Benth., like that of *Asphodelus ramosus*, does not externally suggest a phyllodic anatomy, but it is found to include both normal and inverted bundles. Fig. 13 A, p. 451, shows the transverse section of the leaf near its apex, while Fig. 6, p. 479 of my previous paper,<sup>1</sup> represents the structure of the main part of the leaf. The most striking feature of the leaf of *Eremurus himalaicus* is that there can scarcely be said to be, anatomically, any distinct leaf-sheath region—assuming the absence of inverted bundles to be one of the marks of a sheath. Serial sections through the stem apex show that both normal and inverted bundles continue to the extreme base of the leaf. This is also the case with the sheathing scale leaves which clothe the leaf-bud externally. In these the inverted bundles persist to the base, though they are less numerous than in the foliage leaves (Fig. 13 B). It is possible to take the view that in *Eremurus* there is more or less complete fusion between the leaf-base and the axis. On the other hand, it must be conceded that we need not necessarily exclude an organ from the category of leaf-sheaths or leaf-bases because of its possession of inverted bundles; for some years ago Worsdell<sup>2</sup> pointed out that, in *Thalictrum flavum*, L., the inverted bundles characteristic of the petiole persist downwards at least into the upper part of the leaf-sheath region. I have been able to confirm this, and the occurrence of these inverted bundles (*i.b.*) is indicated in Fig. 12.

### III. THE PETIOLAR PHYLLODES OF THE JOHNSONIEAE (ASPHODELOIDEAE).

The Johnsonieae are a group of highly xerophilous Australian Liliaceae. Schulze<sup>3</sup> gave some account of their leaf structure in his general work on the anatomy of the Family, but as his descriptions suggest a definitely phyllodic type of structure, and as he paid more attention to minute histological detail than to the general features of the vascular system, it seemed worth while to make a further study of the Tribe. I have been able to examine, in the herbarium of the Cambridge Botany School, material of leaves representing six of the seven genera of Johnsonieae (*Johnsonia*, *Arnocrinum*, *Laxmannia*, *Borya*, *Alania*, and *Sowerbeia*), *Starwelia* being the only one which was inaccessible.

The main feature of the leaf anatomy of the Johnsonieae, as Schulze<sup>4</sup> points out, is a tendency towards the aggregation of the bundles into a central vascular cylinder, enclosed in a common parenchymatous sheath. The structure thus produced seems to me to be strongly reminiscent of petiolar anatomy, and the Johnsonieae thus appear to offer a particularly

<sup>1</sup> Arber, A. (1918).

<sup>3</sup> Schulze, R. (1893).

<sup>2</sup> Worsdell, W. C. (1908).

<sup>4</sup> Ibid.

clear case of phyllody. *Sowerbeia juncea*, Sm., is a typical example. As the accompanying diagrams (Figs. 14 A-C, p. 454) show, three separate vascular strands traverse the leaf-sheath, but in the limb of the leaf, which is more or less triangular in section, there is a ring of bundles embedded in fibres. The structure essentially recalls that of one of the simpler leaves of *Asphodelus liburnicus* (in which the inverted adaxial bundle has not branched), but differs from it in the aggregation of the bundles within a common sheath. Fig. 14 B may also be compared with the transverse section of certain Dicotyledonous petioles such as that of *Clematis Vitalba*<sup>1</sup> (Fig. 14 D).

*Sowerbeia laxiflora*, Lindl., as Schulze<sup>2</sup> has pointed out, differs from the other members of the genus in retaining three distinct bundles in the limb. He speaks of the lateral bundles as being directed in an unusual sense, with the xylem pointing outwards.<sup>3</sup> My sections, however, show an orientation which is the reverse of that which he describes—the xylem of the laterals being directed towards the midrib (Fig. 15). This placing of the lateral bundles corresponds to that in the sheath of *Arnocrinum Drummondii* (Fig. 16 A). Besides the three main bundles to which he refers, I have found, in the limb, several very small additional bundles (*b*. in Fig. 15); I have seen as many as six of these in one transverse section. I have not been able to trace their origin in detail, but their position suggests that they arise as branches of the main bundles. They correspond to the small bundles of *Arnocrinum* and *Laxmannia* (*b*<sub>2</sub>, *b*<sub>3</sub>, *b*<sub>4</sub>, in Figs. 20 and 21).

In *Laxmannia grandiflora*, Lindl., three bundles again enter the leaf-sheath (Fig. 19 A), which is continued upwards into a distinct free ligule (*lig.* in Fig. 19 B). In the limb, in which the bundles are aggregated into an axial strand, the development of fibres reaches a most unusual pitch. The stele of the leaf is shown in Fig. 19 C and on a larger scale in Fig. 21; it will be recognized that, in the case of the principal bundle, *all* the elements—with the exception of the somewhat attenuated V of xylem, and a tiny patch of thin-walled cells on the inner side of the apex of each arm of the V—have become strongly thickened. The treatment necessary in preparing herbarium material for sectioning may possibly have exaggerated the width of the walls, but that the elements in question were, in fact, thick-walled fibres admits of no doubt.

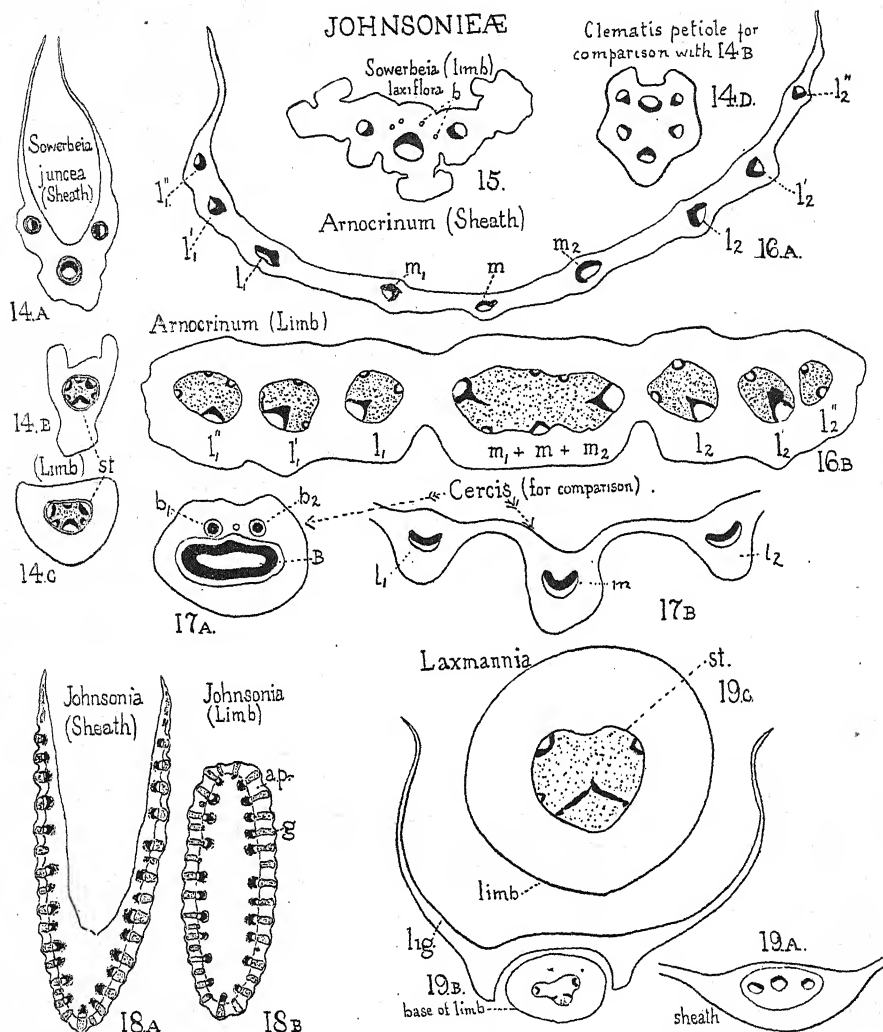
In the genera *Alania* and *Borya*, the vascular system is so much reduced that it appears, in the limb, as Schulze points out, to consist of a single bundle only.

Schulze mentions that he was unable to obtain material of the leaves of *Arnocrinum*, so I have studied the structural plan of *A. Drummondii*, Endl., as fully as I could from the two or three more or less complete

<sup>1</sup> Petit, L. (1887), figures the petiole of this species.

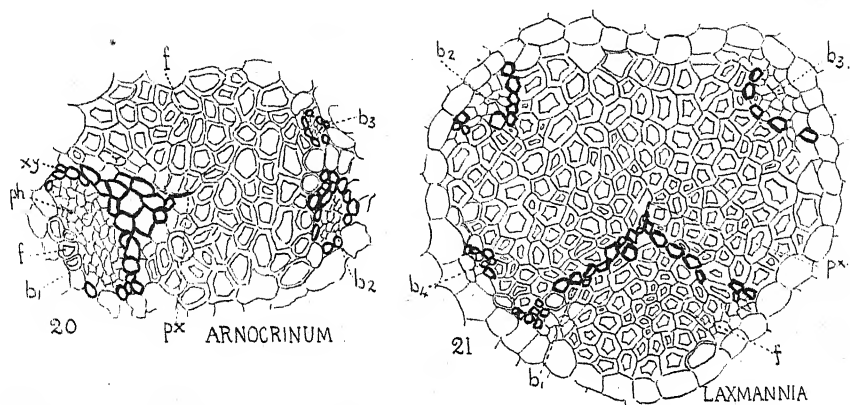
<sup>2</sup> Schulze, R. (1893).

<sup>3</sup> *Ibid.*, p. 334.



FIGS. 14-19. Leaf structure of Johnsonieae. (Xylem, black; phloem, white; fibres, dotted.) Figs. 14 A-C, *Sowerbeia juncea*, Sm. Fig. 14 A, transverse section through leaf-sheath. Figs. 14 B and C, transverse sections through limb; *st.* = axial bundle-group. (All  $\times 14$ .) Fig. 14 D, *Clematis Vitalba*, L. Transverse section of petiole for comparison with limb of *Sowerbeia juncea*, Fig. 14 C ( $\times 14$ ). Fig. 15, *Sowerbeia laxiflora*, Lindl. Transverse section of limb ( $\times 23$ ); *b.* = small additional bundles. Fig. 16 A and B, *Arnocrinum Drummondii*, Endl. Fig. 16 A, transverse section of leaf-sheath ( $\times 14$ );  $l_1$ ,  $l'_1$ ,  $l''_1$ ,  $l_2$ ,  $l'_2$ ,  $l''_2$ ,  $m_1$ ,  $m_2$  = lateral bundles;  $m$  = midrib. Fig. 16 B, transverse section of limb of another leaf ( $\times 14$ ); lettering corresponds to Fig. 16 A (see Fig. 20, p. 455, for one bundle-group on a larger scale). Fig. 17 A and B, *Cercis Siliquastrum*, L., for comparison with *Arnocrinum*. Fig. 17 A, transverse section of petiole, showing steles B,  $b_1$ , and  $b_2$  ( $\times 14$ ). Fig. 17 B, transverse section of base of lamina including midrib,  $m$ , and two main laterals,  $l_1$  and  $l_2$  ( $\times 14$ ). Figs. 18 A and B, *Johnsonia lupulina*, R. Br. Fig. 18 A, transverse section of leaf-sheath ( $\times 14$ ); *g.* = fibrous girder; *ap.* = assimilating parenchyma. Figs. 19 A-C, *Laxmannia grandiflora*, Lindl. Figs. 19 A and B, transverse sections in sheath region ( $\times 14$ ); *lig.* = ligule. In Fig. 19 A the wings of the sheath are omitted. Fig. 19 C, transverse section of the limb ( $\times 23$ ). For a more highly magnified drawing of the central bundle-group (*st.*) see Fig. 21, p. 455.

leaves at my disposal. In the only leaf-base which I was able to section, I found a single series of nine bundles; the midrib was normally orientated, but there was a tendency for the laterals to be placed with their protoxylem pointing towards the midrib (Fig. 16 A). At this level there were no fibres, but higher up each bundle became associated with a group of sclerised elements. Higher still, in the limb itself (Fig. 16 B), the three laterals on either side ( $l_1$ ,  $l'_1$ ,  $l''_1$ , and  $l_2$ ,  $l'_2$ ,  $l''_2$ ) were converted—presumably by branching—into three bundle-groups, while the three bundles,  $m$ ,  $m_1$ , and  $m_2$ , became associated into a central group,  $m$ , which also included a few small additional bundles, in all probability derived by branching from the original strands. Each bundle-group was embedded in fibres (Fig. 20).



FIGS. 20 and 21. Johnsonieae. Fig. 20, *Arnocrinum Drummondii*, Endl. A lateral bundle-group from a section similar to that drawn in Fig. 16 B. It shows a group similar to  $l_1$ , including the three bundles,  $b_1$ ,  $b_2$ , and  $b_3$ , embedded in fibres,  $f$ ;  $xy$ . = xylem;  $ph$ . = phloem;  $px$ . = protoxylem ( $\times 250$ , circa). Fig. 21, *Laxmannia grandiflora*, Lindl. Transverse section of central strand ( $st.$ ) of leaf shown in Fig. 19 C. Lettering as in Fig. 20 ( $\times 150$ , circa).

The most significant feature in the leaf structure of *Arnocrinum* is the fact that the limb—though not the sheath—thus shows polystely.<sup>1</sup> This appears to me to have some bearing upon the 'petiolar phyllode' interpretation of this leaf. Though polystelic petioles do not seem to be common, Petit<sup>2</sup> has drawn attention to certain cases. One of these, *Cercis Siliquastrum*, L., the Judas Tree, I have examined for comparison with *Arnocrinum*. The petiole of *Cercis* contains one large bundle-group and two or more smaller ones ( $B$ ,  $b_1$ ,  $b_2$  in Fig. 17 A), but in the midrib and main laterals of the lamina there are arcs of vascular tissue and all trace of 'polystely' has vanished (Fig. 17 B). Though no great stress must be laid on this comparison, it seems to me that it may be held to indicate that the 'polystely' of *Arnocrinum* is likely to be a petiolar rather than a 'blade' character.

<sup>1</sup> This term is used in a purely descriptive sense.

<sup>2</sup> Petit, L. (1887).



The genus *Johnsonia*, which gives its name to the Tribe, differs rather strikingly from the other members in its leaf structure and anatomy, since it is a typical isobilateral equitant leaf (Fig. 18 A and B), recalling a number of Iridaceae, &c., some of which were figured in a previous paper.<sup>1</sup> Its interest from the standpoint of the Phyllode Theory is that it furnishes an instance of an isobilateral equitant leaf within a Tribe which also includes genera characterized by other types of phyllodic leaf. The same thing occurs, as I have already pointed out,<sup>2</sup> in the Iridoideae and even within the genus *Iris*. The additional case of *Johnsonia* seems to lend colour to the view that the isobilateral equitant leaf should not be interpreted as a case of congenital concrescence, but that it is merely a special type of petiolar phyllode.

#### IV. THE LEAVES OF *ALLIUM* AND *BRODIAEA* (ALLIOIDEAE).

The majority of the records of phyllodic anatomy among the Liliaceae relate to genera belonging to the large Tribe of the Asphodeloideae, which possibly represents that group within the family which has retained the most primitive characters. The other two main Tribes of the Liliaceae proper are the Allioideae and Lilioideae. The Lilioideae—although they include certain leaves which I have interpreted as leaf-base phyllodes terminating in a vestigial petiole<sup>3</sup>—do not apparently present any instances of typical phyllodic anatomy. The Allioideae, however, include a number of cases coming under this head. I propose to consider the genus *Allium* (as representing this Tribe) in some little detail, and to add a brief description of one species of the related genus *Brodiaea* for comparison.

In the case of *Allium* I have examined the leaf structure of at least one species belonging to each Section of the genus; viz.:

##### Section I. PORRUM, G. Don.

*Allium Porrum*, L. (Figs. 22 A-C).

*A. Scorodoprasum*, L.

*A. Ampeloprasum*, L.

##### Section II. SCHOENOPRASUM, G. Don.

*A. Schoenoprasum*, L. (Figs. 24 A and B).

*A. fistulosum*, L. (Figs. 23 A-D).

##### Section III. RHIZIRIDIUM, G. Don.

*A. victorialis*, L. (Figs. 25 A and B).

##### Section IV. MACROSPATHA, G. Don.

*A. carinatum*, L. (Figs. 26 A and B).

##### Section V. MOLIMUM, G. Don.

*A. ursinum*, L. (Figs. 28 A-E).

*A. Chamaemoly*, L.

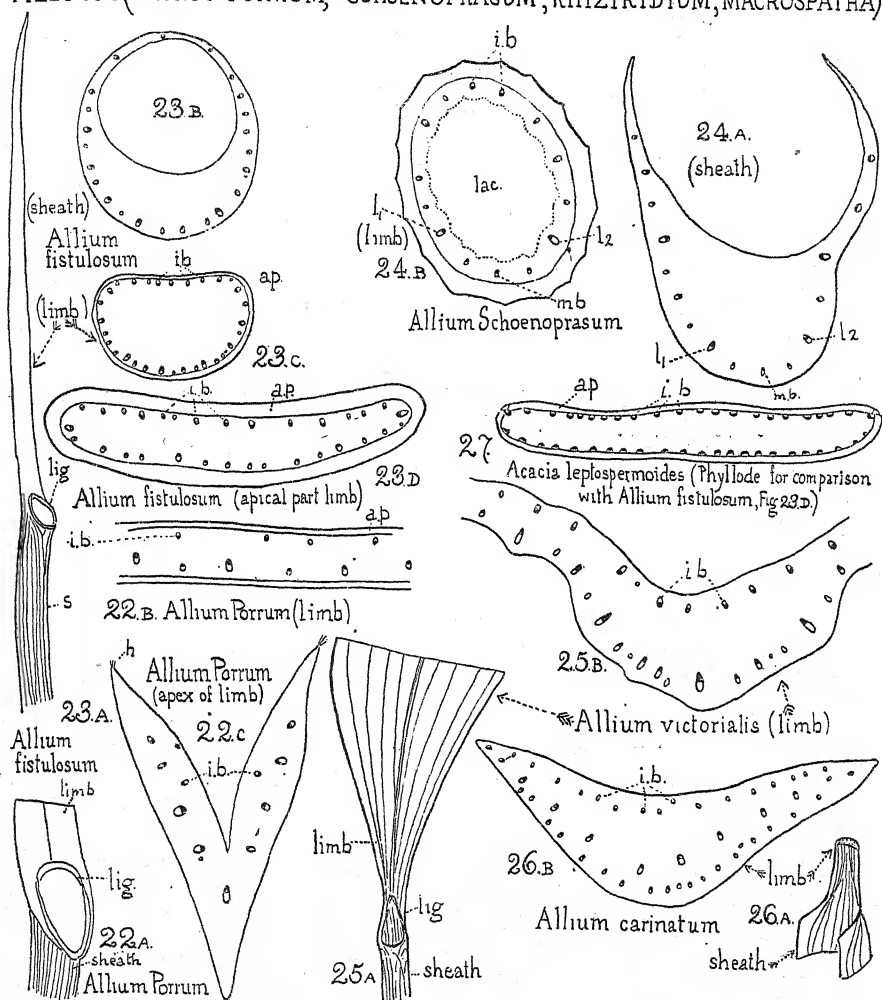
*A. Moly*, L. (Figs. 29 A-C).

<sup>1</sup> Arber, A. (1918), p. 483.

<sup>2</sup> Ibid., pp. 484-5.

<sup>3</sup> Ibid. (1920<sup>1</sup>).

## ALLIUM (SECTIONS—PORRUM, SCHOENOPRASUM, RHIZIRIDIUM, MACROSPATHA)



FIGS. 22-27. Leaf structure of *Allium* (xylem, black; phloem, white). Figs. 22 A-C, *Allium Porrum*, L. (Sect. *Porrum*). Fig. 22 A, junction of sheath and limb, with ligule, *lig.* ( $\frac{1}{2}$  nat. size). Fig. 22 B, transverse section of part of limb, not including midrib ( $\times 9$ , circa); *i.b.* = inverted bundle; *a.p.* = assimilating parenchyma. Fig. 22 C, transverse section close to apex of limb, to show survival of inverted bundles, *i.b.*, in this region; *h.* = marginal hairs ( $\times 23$ ). Figs. 23 A-D, *Allium fistulosum*, L. (Sect. *Schoenoprasum*). Fig. 23 A, leaf ( $\frac{1}{2}$  nat. size) to show upper part of sheath, *s.*, ligule, *lig.*, and limb. Fig. 23 B, transverse section of sheath ( $\times 5\frac{1}{2}$ , circa). Fig. 23 C, transverse section of limb ( $\times 5\frac{1}{2}$ , circa); *i.b.* = inverted bundle. Fig. 23 D, transverse section of flattened apical part of limb ( $\times 14$ ). Figs. 24 A and B, *Allium Schoenoprasum*, L. Fig. 24 A, transverse section of sheath ( $\times 23$ ). Fig. 24 B, transverse section of limb ( $\times 23$ ). (Note in both cases relative unimportance of midrib, *m.b.*, as compared with main laterals, *l.* and *l.*) Figs. 25 A and B, *Allium victorialis*, L. (Sect. *Rhiziridium*). Fig. 25 A, junction of limb and sheath showing ligule, *lig.* ( $\frac{1}{2}$  nat. size). Fig. 25 B, transverse section of midrib region of limb; *i.b.* = inverted bundle ( $\times 8\frac{1}{2}$ , circa). Figs. 26 A and B, *Allium carinatum*, L. (Sect. *Macrospatha*). Fig. 26 A, junction of sheath and limb ( $\frac{1}{2}$  nat. size). Fig. 26 B, transverse section of limb near its junction with sheath ( $\times 8\frac{1}{2}$ , circa). Fig. 27, *Acacia leptospermoides*, Benth. Transverse section of phyllode ( $\times 14$ ) for comparison with *Allium fistulosum* (Fig. 23 D); *a.p.* = assimilating parenchyma; *i.b.* = inverted bundles.

## Section VI. NECTAROSCORDUM, Lindl.

*A. Dioscoridis*, Sibth. et Sm. (Fig. 30).

## Section VII. MICROSCORDUM, Maxim.

*A. Monanthum*, Maxim. (Fig. 31).

There is a strong general similarity between those members of the first three Sections which I have been able to examine. The leaf is differentiated into a basal sheath and a definite limb, the boundary between these two regions being marked by a distinct ligule (Figs. 22 A, 23 A, 25 A). The limb may be linear and flattened as in *A. Porrum* (Fig. 22 A and B); or broad and flattened as in *A. victorialis* (Fig. 25 A); or tubular and more or less semicircular in section as in *A. fistulosum* (Fig. 23 A and C). The peculiar hollow leaves of certain Onions have attracted the attention of botanists from the earliest days; their existence was recorded by Theophrastus<sup>1</sup> (born 370 B.C.). In all the cases which I have examined in Sections I, II, and III, the anatomy of the limb—whether flattened or cylindrical—is definitely phyllodic, with inverted as well as normal bundles (*i.e.* in Figs. 22 B and C, 23 C, 24 B, and 25 B). The flattened apical region of the limb of *A. fistulosum* (Fig. 23 D) may be closely compared with the phyllode of *Acacia leptospermoides*, Benth. (Fig. 27), which is unusual for that genus in being expanded in the horizontal plane.<sup>2</sup>

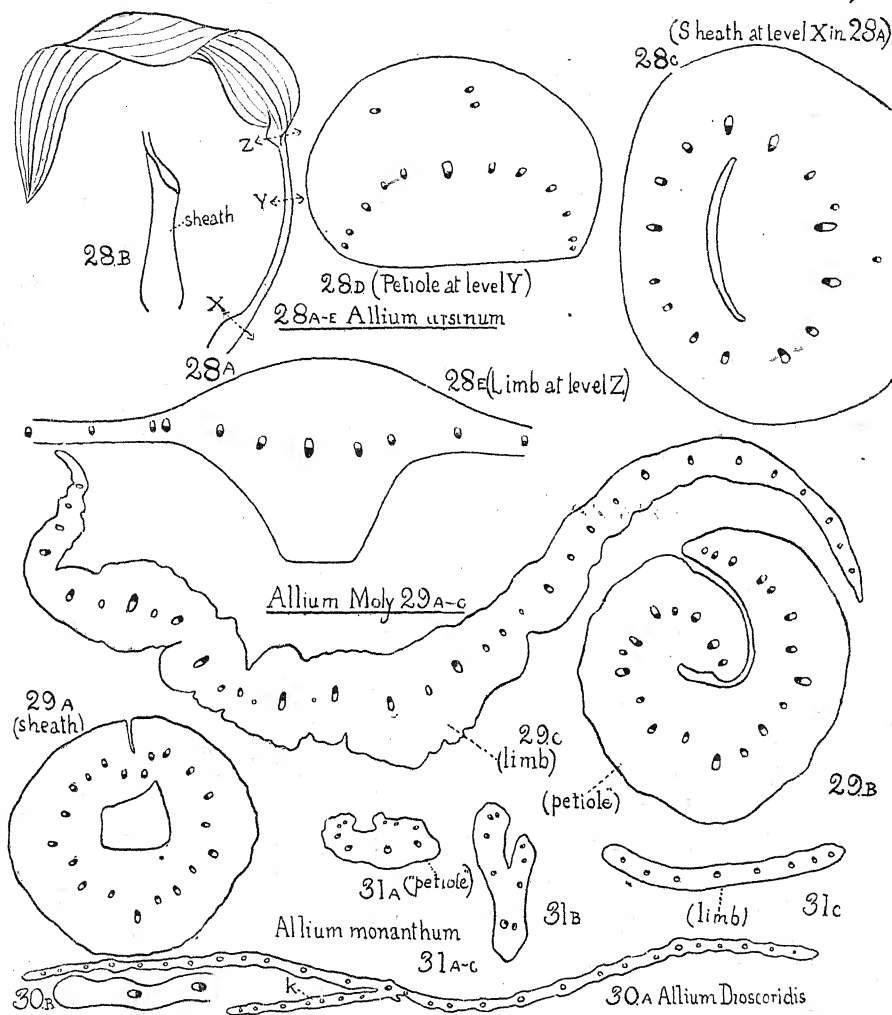
*Allium carinatum*, the only member of Section IV (*Macrospatha*) which I have studied, differs slightly from the species hitherto mentioned in not possessing a ligule, but there is a sharp distinction between the limb and the sheath with its membranous wings (Fig. 26 A). The usual inverted bundles occur in the limb (Fig. 26 B).

As far as my examination goes, I should say that in Sections I–IV of the genus we have leaves which include both leaf-sheath and limb, the latter being a *petiolar phyllode*. Section V (*Molium*) is more puzzling. In *Allium Moly*, the Lily Leek, there is a sheath, which is swollen at the base, then a slender region looking externally like a petiole, and then a relatively broad limb. But sections reveal the fact that the ‘petiole’ (Fig. 29 B) is not a solid structure, but is merely the upward continuation of the rolled leaf-sheath (Fig. 29 A), and that the ‘blade’ (Fig. 29 C) is also nothing but a direct prolongation and expansion of the sheath. Any sharp distinction between sheath, petiole, and blade seems to be purely arbitrary. The blade is non-phyllodic in anatomy, containing a single series of bundles (Fig. 29 C). The blade of *A. Chamaemoly* is also similar in structure. The curious inverted limb of *Allium ursinum* (Fig. 28) also shows no trace of phyllodic anatomy (Fig. 28 E), while the petiole with its single arc of bundles (Fig. 28 C) looks as if it corresponded to the dorsal side of the sheath.

<sup>1</sup> Theophrastus: ‘Enquiry into Plants,’ trans. by A. Hort. Loeb’s Classical Library, 1916, vol. i, p. 77.

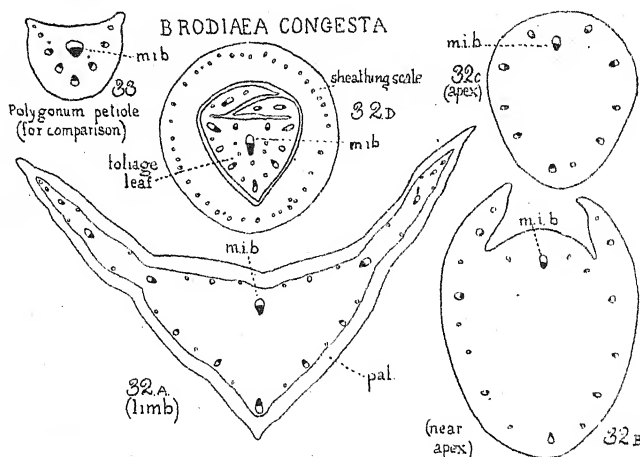
<sup>2</sup> Hochreutiner, G. (1896).

## ALLIUM (SECTIONS—MOLIUM, NECTAROSCORDUM MICROSCORDUM)



FIGS. 28-31. Leaf structure of *Allium*. Figs. 28A-E, *Allium ursinum*, L. (Sect. *MOLIUM*). Fig. 28A, leaf of non-flowering plant ( $\frac{1}{2}$  nat. size). Fig. 28B, sheathing base of leaf of flowering plant ( $\frac{1}{2}$  nat. size). Fig. 28C, transverse section of sheath at level X; Fig. 28D, transverse section of petiole at level Y; Fig. 28E, transverse section of limb at level Z (Figs. 28C, D, E,  $\times 14$ ). (Note partial twisting in Fig. 28C, and inversion in Figs. 28D and E.) Figs. 29A-C, *Allium Moly*, L. (Sect. *MOLIUM*). Fig. 29A, sheath just above the great swelling which forms the bulb; Fig. 29B, apparent petiole; Fig. 29C, limb of leaf. (All  $\times 14$ .) Fig. 30A and B, *Allium Dioscoridis*, Sibth. et Sm. (Sect. *NECTAROSCORDUM*). Fig. 30A, transverse section of limb of leaf ( $\times 14$ ); k = keel. Fig. 30B, margin of limb in Fig. 30A, further enlarged to show orientation of marginal bundles ( $\times 47$ ); Figs. 31A-C, *Allium monanthum*, Maxim. Fig. 31A, transverse section of apparent petiole; Fig. 31B, transverse section of intermediate region; Fig. 31C, transverse section of base of limb. (All  $\times 14$ .) There is probably a good deal of distortion in Figs. 30 and 31, due to imperfect recovery of form of the herbarium material used.

Judging from the three species which I have examined, I am disposed to think that there is so sharp a difference in leaf morphology and anatomy between the Section *Molium* and the *Alliums* belonging to the preceding Sections that it is conceivable that *Molium* deserves elevation into a distinct genus, or even that it might be well to treat both *A. ursinum* and *A. Moly* as generic types.<sup>1</sup> However this may be, it certainly seems that it is difficult to explain the leaves of this Section on the same lines as those of Sections I-IV. The most probable view appears to me to be that the leaves of *Allium Moly*, *A. Chamaemoly*, and *A. ursinum* do not, like the



FIGS. 32 and 33. Figs. 32 A-D, *Brodiaea congesta*, Sm. (xylem, black; phloem, white; *m.i.b.* = main inverted bundle). Fig. 32 A, transverse section of limb of leaf; *pal.* = palisade parenchyma ( $\times 11$ ). Fig. 32 B, transverse section of another leaf near apex ( $\times 18$ ). Fig. 32 C, transverse section close to extreme apex ( $\times 18$ ). Fig. 32 D, base of sheathing leaf and first foliage leaf of young vegetative shoot ( $\times 11$ ). Fig. 33, *Polygonum amphibium*, L. Transverse section of petiole for comparison with limb of *Brodiaea*; *m.i.b.* = main inverted bundle ( $\times 11$ ).

typical *Alliums*, consist of leaf-base and petiole, but are reduced to leaf-base alone, and that their 'laminae' are merely expansions of the upper part of this leaf-sheath. On this view the *Molium* Section would possess a more reduced and advanced type of leaf than the rest of the genus. If this hypothesis holds good, we shall expect to find that the *Alliums* with the widest geographical distribution occur in other Sections, rather than in the Section *Molium*. This expectation is, as a matter of fact, realized, for no member of the *Molium* Section extends into the New World, whereas *A. Schoenoprasum*<sup>2</sup> and *A. victorialis*, with their phyllodic leaves, occur not only in Europe and Asia, but also in North America.

<sup>1</sup> Irmisch, T. (1850), shows that *A. ursinum* differs markedly from *A. Moly* in its general morphology.

<sup>2</sup> In this connexion it may be mentioned that Lampa, E. (1900), has put forward the general view that in the Liliaceae the 'Rundblatt' is primitive. This writer does not allude to the possibility of interpreting the Monocotyledonous leaf in terms of the Phyllode Theory, but the

Of *Allium Dioscoridis*, Sibth. et Sm. (Section *Nectaroscordum*), I have only been able to examine one small piece of the limb of a leaf from Sicily. The structure is sufficiently striking—the limb is thin and is furnished with a single series of bundles, but from the midrib region a plate-like keel originates (k, Fig. 30 A). The herbarium material at my disposal did not enable me to satisfy myself about the orientation of the bundles, except those on the margins of the limb, which are placed horizontally (Fig. 30 B; see also pp. 463-4). This account must be considered as purely provisional; I hope to get further material and to study the peculiar structure of this leaf in detail. Its ground-plan appears to recall that of certain Iridaceous leaves, but it remains to be seen whether this comparison can be maintained.

In the case of *Allium Monanthum*, Maxim. (Sect. *Microscordum*), I have again not been able, owing to paucity of material, to examine the structure adequately. The fragment of a leaf from Japan, which I sectioned, showed, however, a general similarity to that of *A. Moly*. At the base there was an apparent petiole (Fig. 31 A), probably of sheath nature, while the limb had one series of normally orientated bundles (Fig. 31 C).

*Brodiaea congesta*, Sm., another member of the Alliaceae, has scale leaves with a single row of normally orientated bundles (Fig. 32 D), and also phyllodic foliage leaves with inverted as well as normal bundles (Figs. 32 A-D). I regard the latter as petiolar, and the former as of leaf-base nature. At the extreme apex, the foliage leaf becomes almost cylindrical (Fig. 32 C). The most striking feature of the anatomy is the presence of a median inverted bundle which is larger than the midrib (*m.i.b.* in Figs. 32 A-D). This peculiarity can be paralleled in the petioles of certain Polygonaceae, e.g. *Polygonum amphibium*, L. (Fig. 33), and *Antigonon leptopus*, Hook. et Arn. (Fig. 35). This median inverted strand must not be claimed, however, as an exclusively petiolar character, since in *Polygonum amphibium* it persists into the midrib.

#### V. THE LEAVES OF *ASTELIA* AND *DASYLIRION* (DRACAENOIDEAE).

Predominance of the main laterals, associated with relative insignificance of the median bundle—a somewhat different thing from the lack of well-defined symmetry about a midrib referred to on p. 449—is a noticeable character of the leaf of certain members of the genus *Astelia* (Dracaenoideae). I have seen it in sections of *A. Solandri*, A. Cunn. (Fig. 34), and *A. Banksii*, A. Cunn., and, judging from the external appearance, the same thing occurs in *A. grandis*, Hook. f., and *A. trinervia*, T. Kirk. In *A. alpina*, R. Br., on the other hand, the three main strands are almost equal in size. A similar small midrib with large main laterals occurs in *Allium Schoenopariarum*. The peculiarities of leaf structure to which she draws attention are precisely those on which this theory throws light.

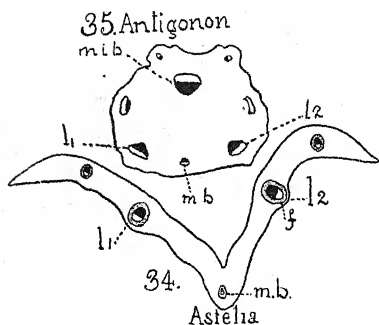
*prasum* (Figs. 24 A and B, p. 457), while in *Arnocrinum Drummondii* (Fig. 16 A, p. 454) the median bundle is less well developed than the laterals on either side of it. In the isobilateral equitant leaf of *Tritonia* (Iridaceae) the main laterals are again the predominating strands.<sup>1</sup> I know of no parallel for the condition in *A. Solandri*, &c., among Dicotyledonous laminae, but the petiolar phyllodes of certain Acacias show just the same relation of a small median bundle to large main laterals.<sup>2</sup> Though the great majority of petioles have a midrib, Petit<sup>3</sup> has drawn attention to its absence in certain cases, and its relative insignificance in others. In the petiole of *Antigonon leptopus*, Hook. et Arn., for instance, the midrib bears

much the same relation to the laterals as in *Astelia* (Fig. 35). It seems to me possible that, in emphasizing its main laterals rather than its midrib, the leaf of *Astelia* is revealing a symptom which would more readily develop in a phyllode—whether of leaf-base or petiolar nature—than in a true lamina.

Through the kindness of Dr. Greenman, of the Missouri Botanical Garden, I have been able to examine the leaf anatomy of a series of species of *Dasyllirion*: *D. acrostichum*, Zucc., *D. cedrosanum*, Trelease, *D. glaucophyllum*, Hook., *D. graminifolium*, Zucc., *D. leiophyllum*, Engelm., *D. longissimum*, Lem., *D. lucidum*, Rose,

*D. Palmeri*, Trelease, *D. serratifolium*, Zucc., *D. texanum*, Scheele, and *D. Wheeleri*, S. Wats.

*Dasyllirion longissimum* (Fig. 36) has a centric leaf, but all the other species enumerated are more or less flattened, e.g. *D. Palmeri* (Fig. 37). The anatomy of the limb of the leaf is essentially uniform throughout these eleven species and all the main bundles are normally orientated; though some of the smaller bundles are irregularly placed, there is no series of strands with inverted orientation. In general there is a series of large bundles (*b* I) lying towards the upper surface, and a series of smaller ones towards the lower surface (*b* II). Sometimes a third series of smaller bundles (*b* III) lies close to the upper surface (e.g. *D. longissimum*, Fig. 36). There may be a number of irregularly orientated bundles (*b* IV) in the parenchyma in the middle of the leaf, and also a number of similar strands,



FIGS. 34 and 35. Fig. 34, *Astelia Solandri*, A. Cunn. Transverse section of limb of leaf ( $\times 23$ ); *m.b.*, median bundle; *l1* and *l2*, laterals; *f.*, fibres. Fig. 35, *Antigonon leptopus*, Hook. et Arn. (Polygonaceae). Transverse section of petiole for comparison with *Astelia*, showing small size of main bundle (*m.b.*) in comparison with the laterals (*l1* and *l2*); *m.i.b.* = main inverted bundle. ( $\times 14$ .)

<sup>1</sup> Arber, A. (1918), Fig. 15 B, p. 483.

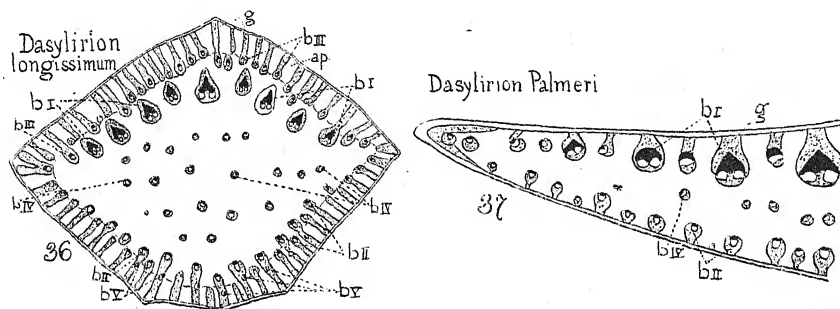
<sup>2</sup> Ibid., Figs. 2 B, C, D, p. 474.

<sup>3</sup> Petit (1887 and 1889).



often lying sideways, between the lower series of small bundles and the lower margin of the leaf (*b v*). The great development of fibrous girders (*g.*) is very characteristic and the bundles of series *b v* are apt to be embedded in them.

The absence of phyllodic anatomy in the Dasyliirions is exactly what might have been anticipated. The Dracaenoideae, with their tendency towards the tree habit, probably represent an advanced and specialized



FIGS. 36 and 37. *Dasyliirion* (xylem, black; phloem, white; fibres, dotted). Fig. 36, transverse section of limb of leaf of *D. longissimum*, Lem. ( $\times 12\frac{1}{2}$ , circa); *b i-b v*, bundles belonging to different series (see text); *g.* fibrous girder; *a.p.*, assimilating parenchyma. Fig. 37, transverse section of leaf margin of *D. Palmeri*, Trelease ( $\times 12\frac{1}{2}$ , circa). Lettering as in Fig. 36.

group of the Liliaceae. Writing of the sub-tribe Nolineae, to which the Dasyliirions belong, Trelease<sup>1</sup> says, 'No reason is apparent for considering it to be very ancient'. The xerophytic type of leaf of the Dasyliirions, with more than one series of bundles, all normally orientated, may be contrasted with the phyllodic anatomy of those externally similar xerophytic leaves belonging to that more primitive Tribe, the Asphodeloideae; *Dasyliirion longissimum* (Fig. 36) may be set beside *Xanthorrhoea*,<sup>2</sup> while *D. Palmeri* (Fig. 37) offers a similar contrast to *Asphodelus ramosus* (Fig. 8).<sup>3</sup>

#### VI. THE LEAF ANATOMY OF *OPHIOPOGON* (OPHIOPOGONOIDEAE).

It may be well here to draw attention to the structure of the *Ophiopogon* leaf, because it has been claimed by Schulze<sup>4</sup> as exemplifying an anomalous arrangement of xylem and phloem—the xylem of the lateral bundles being described as directed towards the leaf margins. This is no doubt an error due to the extreme fibrosis of the phloem, which makes it look deceptively like wood. Examination of very young leaves of *O. japonicus*, Ker-Gawl (Figs. 38A and B) shows that the lateral bundles are, in reality, placed with the xylem directed obliquely towards the midrib. This somewhat unusual orientation may be paralleled in the lateral bundles of

<sup>1</sup> Trelease, W. (1911).

<sup>2</sup> Arber, A. (1918), Fig. 12, p. 479.

<sup>3</sup> Zuccarini, J. G. (1837-40), regards the leaf of the genus as essentially petiolar; this may be correct, but it appears more probable to me that it is merely a highly differentiated leaf-base.

<sup>4</sup> Schulze, R. (1893).

the limb of *Allium Dioscoridis* (Fig. 30 B, p. 459), the sheath of *Arnocrinum Drummondii* (Fig. 16 A, p. 454) and the limb of *Sowerbeia laxiflora* (Fig. 15, p. 454). It may possibly be regarded as a phyllodic feature, since it

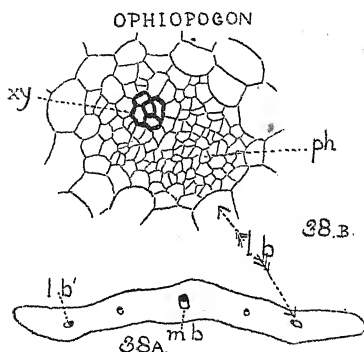


FIG. 38. *Ophiopogon japonicus*, Ker-Gawl. Fig. 38 A, transverse section of young leaf ( $\times 23$ ); *m.b.* = median bundle; *l.b.* and *l.b.'* = main lateral bundles. Fig. 38 B, *l.b.* on a larger scale; *xy.* = xylem; *ph.* = phloem ( $\times 318$ , circa).

characterizes the marginal strands of the phyllodes of *Oxalis bupleurifolia*, A. St. Hil., in which all the remaining bundles form a single normally orientated series.<sup>1</sup>

#### ACKNOWLEDGEMENTS.

I have pleasure in acknowledging my indebtedness for living plants, or herbarium specimens, to Mr. J. H. Maiden, F.R.S., Director of the Botanic Gardens, Sydney; Professor Ikeno, of Tokyo; Dr. J. M. Greenman, Curator of the Herbarium, Missouri Botanic Garden; and—in England—to the Director and to the Keeper of the Herbarium, the Royal Botanic Gardens, Kew; the Curator of the Cambridge Botanic Garden; the Keeper of the Botanical Department, British Museum (Nat. Hist.); and especially to Professor A. C. Seward, F.R.S., for permission to study material in the Herbarium of the Botany School, Cambridge.

I wish also to express my thanks to Miss E. R. Saunders, Director of the Balfour Laboratory, where the present work has been carried out with the aid of a grant from the Dixon Fund of the University of London.

<sup>1</sup> Arber, A. (1918), Fig. 3 B, p. 474.

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# Clathrosorus, a New Genus of Plasmodiophoraceae.

BY

C. FERDINANDSEN

AND

Ö. WINGE.

With Plate XXI.

THE material of the species here described was sent to us from Sorö, Sealand (Denmark), by Mr. H. Gram, consulting horticulturist, in the month of June, 1918. On roguing bluebells (*Campanula rapunculoides*) from his garden Mr. Gram observed that the roots of the named weed often showed numerous small swellings, calling in mind to a certain degree the bacterial tumours on the roots of leguminous plants (see Pl. XXI, Fig. 1). Some root-swellings sent to us for closer investigation were fixed in Carnoy, and as it proved that we had before us a new and interesting Plasmodiophoracea we made some slides of the material for a more detailed examination. Preparations stained with cyanine-gold-orange showed in their cytological features a pretty close accordance with the other Plasmodiophoraceae.<sup>1</sup> We have observed the nuclear divisions in the vegetative stage, whereof Figs. 2 and 3 on Pl. XXI give a pair of pictures, as well as in the akaryotic and sporogonic phase; in the last-named stage we saw both the heterotypic and the homoiotypic division.

The infection falls only on the cortex, whose more peripheric cells nearly all contain a single plurinucleate myxoplasma or a sporosorus; in the direction of the central cylinder the number of uninfested cells increases. Into the strongly distorted central cylinder itself the parasite never comes. The myxoplasma very often is lodging around the nucleus. The cells are scarcely hypertrophied by the invasion, but are stimulated to more frequent divisions; as a rule the myxoplasma, respectively the sporosorus, will not by far fill out the lumen of the invaded cell. It is further characteristic of

<sup>1</sup> See, e.g., Maire et Tisson: La cytologie des Plasmodiophoracées et la classe des Phytomyxinae; Ann. Myc., 1909. Blomfield and Schwartz: Observations on the Tumours on *Veronica Chamaedrys* caused by *Sorosphaera veronicae*; Ann. Bot., 1910. Winge, Ö.: Cytological Studies in the Plasmodiophoraceae; Arkiv för Botanik, 1912.

the parasite that the ripe sorus has the shape of an irregular, rounded or elongated ball with an uneven surface and traversed by larger and smaller cavities. The individual spores are not so solidly connected as, e. g., in *Sorosphaera* and *Sorodiscus*, where the spores are conglutinated with a common substance. In contrast to the case in all hitherto known genera of Plasmodiophoraceae the spores have at the full maturity, but first then, a finely punctuate warty membrane.

We have stated that the spores treated with Carnoy are somewhat collapsing, thus becoming flattened or depressed, and as if provided with a collar, while the spores preserved in diluted alcohol do not show this feature. Most of the other Plasmodiophoraceae seem to behave in the same manner.

Below we give a diagnosis of the new organism.

CLATHROSORUS, gen. nov.

Spec. typ.: *Clathrosorus campanulae*, sp. n.

Plasmodiophoracea radiculicola, tumefaciens, cellulas corticis solum infestans. Amplificatio cellularum vix ulla. Sporosori in singulis cellulis sporosoro non impletis singuli, rotundati vel oblongi, saepe irregulares, clathrato-canaliculati. Sporae strato communi non conglutinatae, maturae globosae vel subglobosae, flavidulae, episporio subtiliter punctato-verruculoso.

CLATHROSORUS CAMPANULAE, sp. nov.

*Statu vegetativo* myxamoebae paulatim plurinucleatae in singulis cellulis corticis aggressu vix auctis singulae inventae, nucleum cellulae hospitalis saepe amplectentes.

*Statu fructifero* sporosori in singulis cellulis sporosoro non impletis singuli, transverse secti clathrato-canaliculati, desuper visi rotundati vel oblongi, foveolati et hinc circuitu irregulari, circ.  $25-50\mu$  lati. Sporae globosae nec non late ellipsoideae, siccitate fluidisve fixativis validis collabescentes, sub vitro gregatim flavae, singulatim hyalino-flavidulae, episporio subtiliter punctato-verruculoso,  $4-5\frac{1}{2}\mu$  diametro.

Ad radices *Campanulae rapunculoidis* quae aggressu parasitae eodem fere modo ac radices Leguminosarum bacilligerae intumescunt, in horto prope Soras Daniae. (Leg. H. Gram.)

EXPLANATION OF PLATE XXI.

Illustrating Messrs. Ferdinandsen and Winge's paper on *Clathrosorus*, a New Genus of Plasmodiophoraceae.

Fig. 1. Appearance of a specimen of *Campanula rapunculoides* infested by *Clathrosorus campanulae*. Nat. size.

Figs. 2 and 3. Young amoebae showing the characteristic nuclear divisions, anaphase and metaphase respectively.  $\times 1,680$ .

Fig. 4. Multinucleate amoeba in a host cell.  $\times 750$ .

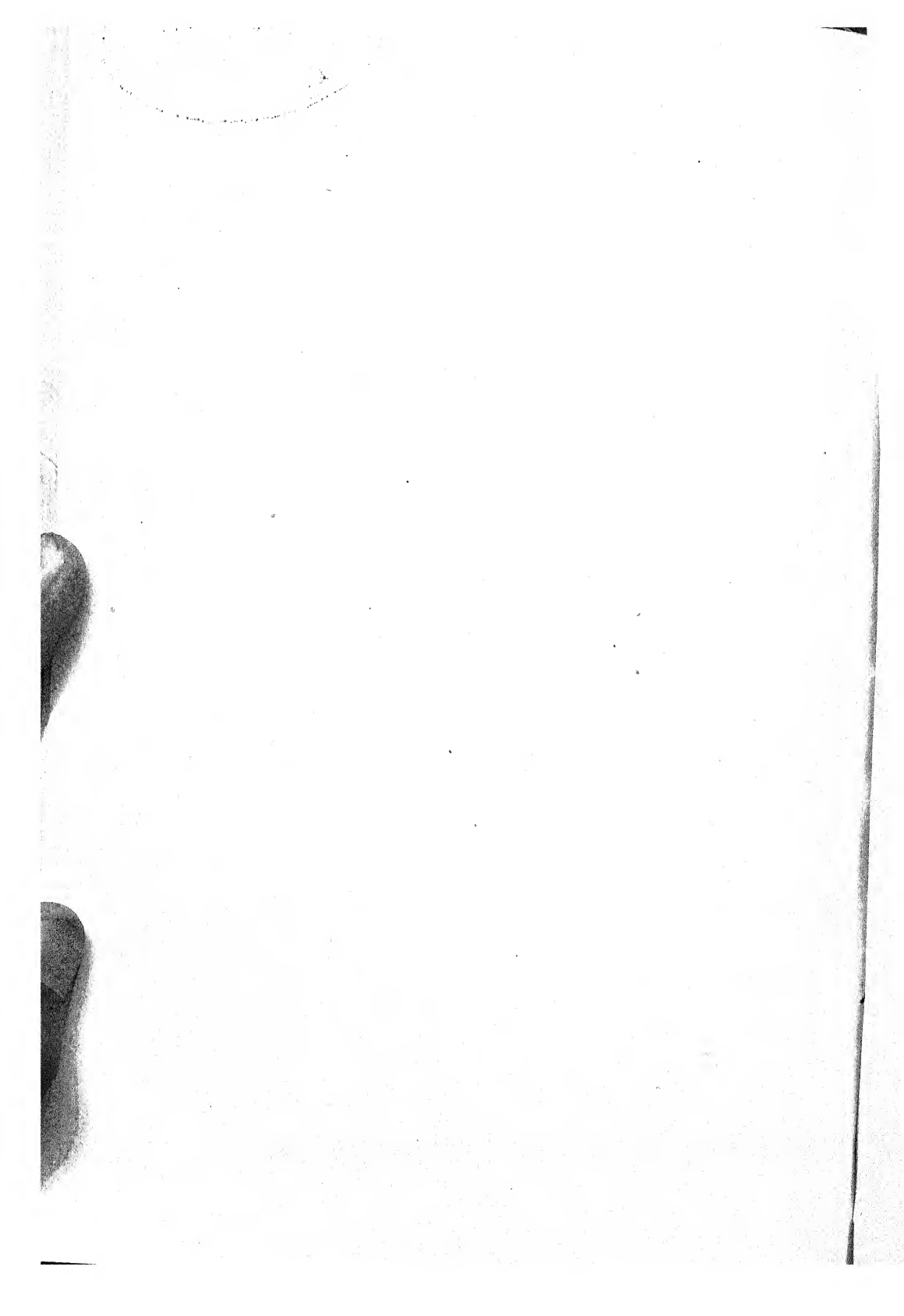
Fig. 5. Multinucleate amoeba surrounding a part of plasma and the nucleus of the host cell.  $\times 750$ .

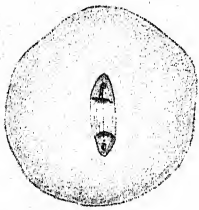
Fig. 6. Aggregation of spores in a host cell.  $\times 570$ .

Fig. 7. Section through a spore ball.  $\times 700$ .

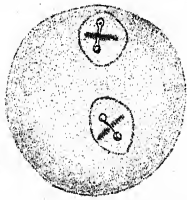
Fig. 8. Section through two mature spores.  $\times 1,680$ .



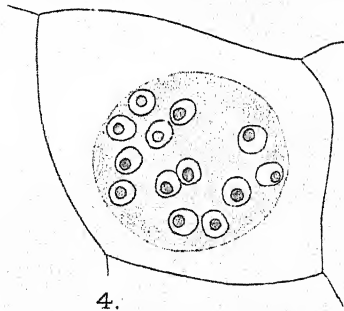




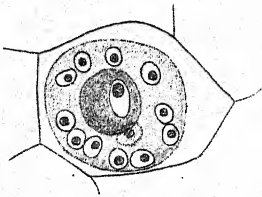
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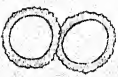
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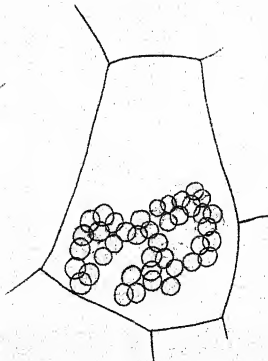
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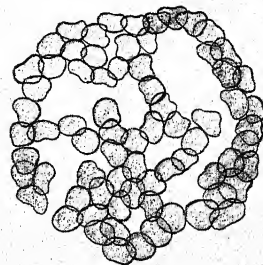
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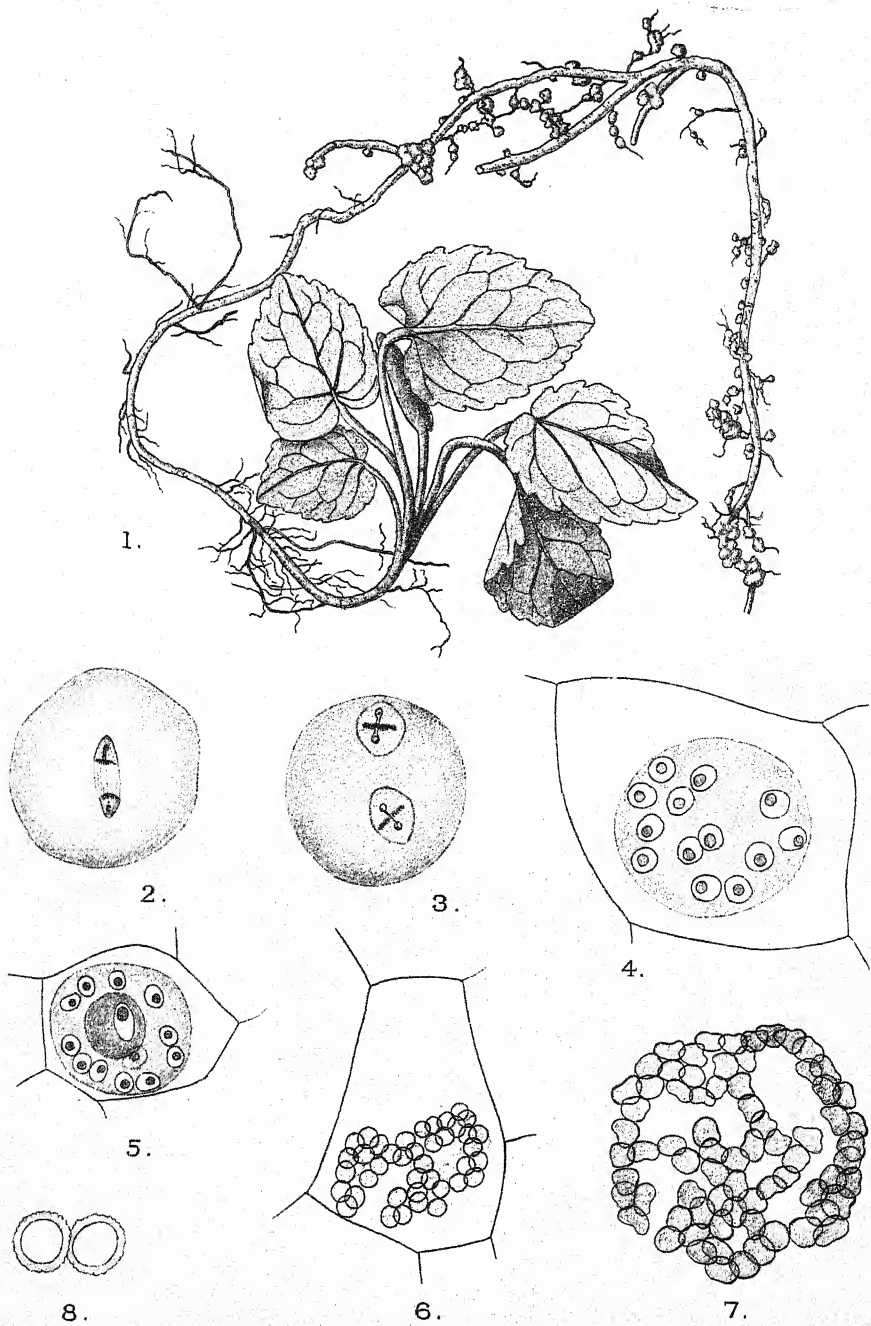
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Huth, London.





Huth, London.



# Plant Invasions of New Zealand with Reference to Lord Howe, Norfolk, and the Kermadec Islands.

BY

J. C. WILLIS, M.A., Sc.D., F.R.S.,

*European Correspondent of the Botanic Garden, Rio de Janeiro.*

With three Diagrams and eleven Tables in the Text.

IN a series of eight papers in this journal, 1916-19, I have dealt with the floras of New Zealand proper and the islands which outlie from it to the north (Kermadecs), east (Chathams), and south (Stewart and Aucklands), islands which must have received a large part of their flora either directly from New Zealand or from invasions which passed near to them on the way thither. In these papers I have made a great many predictions based upon my hypothesis of age and area, and have always found them borne out by the facts. It is almost needless to say that in some cases these facts were already known to New Zealand and other botanists, though very many are new. My chief object in making all these predictions was to marshal the facts and to show (and I venture to think that I have shown) that age and area can be relied upon as a guide in the taxonomic distribution problems of a well-defined area like New Zealand and its immediately outlying islands. I shall now go farther afield, and endeavour to trace farther towards their source some of the invasions of plants which appear to have reached New Zealand from the north or north-west.

One of the principal objects kept in view in this work is to show that the floras of the islands which lie between New Zealand and Australia or Polynesia fit in with my hypothesis of age and area, and are explicable on that hypothesis; further, that they are also capable of being dealt with by aid of numerical methods, like the floras of New Zealand itself, and, therefore, that the invasions of plants must have been by land—casual trans-aqueous carriage would not produce such results.

In the first papers of this series, dealing with Ceylon (7), by means of statistics of actual distribution of the various species, which were taken from Trimen's Flora, and which showed that the least widely distributed species in the island were those confined to Ceylon, the next those confined to Ceylon and South India, and the most widely distributed in the island those

which had a greater distribution abroad than merely to South India, I deduced the conclusion that in a given country the area of distribution of a species (working always with groups of at least ten allied species) depended upon its age in that country. The correctness of this deduction was then confirmed by observations upon the floras of New Zealand and its surrounding islands, which gave exactly parallel results, and also by observations on the floras of Hawaii, Jamaica, &c.

In my first paper on the New Zealand flora (9), I assumed the correctness of the hypothesis (which for convenience I have termed that of 'age and area'), and by means of the diagram here reproduced I predicted the result

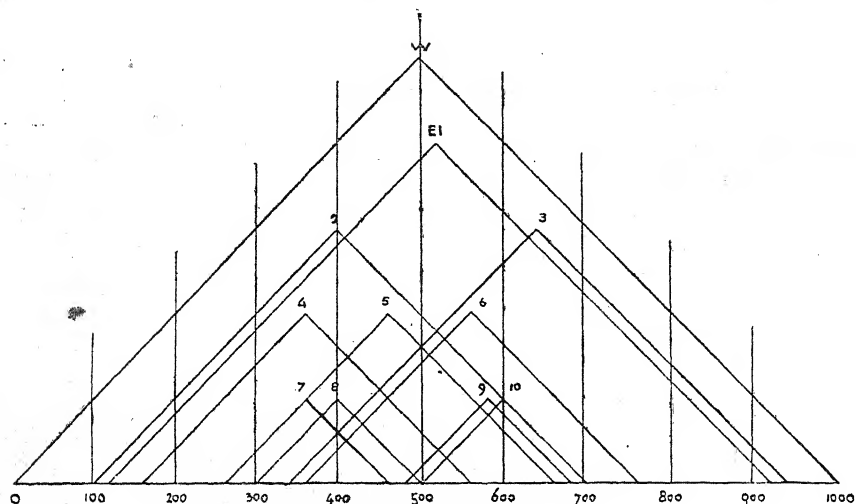


DIAGRAM I.

of the entrance of a single species (W) at the centre of New Zealand, which spread at a uniform rate (represented by the triangle), and which subsequently gave rise to local endemic species (E 1 to E 10) in a casual way. The length of New Zealand, supposed 1,000 miles, being divided into ten zones of equal width, and the number of endemics found in each zone being counted (each endemic of course being supposed to spread uniformly like the parent species, as indicated by the similar triangles), the result shows a curve rising and falling to and from a maximum (sometimes two) which is somewhere near the point of entry of the original species. In the present case, for example, the numbers of endemics in each consecutive zone are—

0    3    5    8    9<sup>1</sup>    8    7    3    2    2

It will be well to make clear at this point that if the entry of the original species be at one end, instead of in the middle of New Zealand, the curve will in general show its maximum at or near that end. The maximum,

<sup>1</sup> The thicker type indicates the maximum in this and the following table.



in other words, is in general at or near the point of entry of the original species. Further, it is not necessary that the entry should be at a point. If it were, for example, by the whole zone from 300 to 700 miles, the resulting curve would be of the same type.

Examination of the actual figures for the distribution of the New Zealand flora soon showed that every genus did in fact give a curve of this type, so that a fact of great significance was thus discovered, and one which may be considered without reference to the hypothesis of age and area, by whose assistance it was originally found. The distribution of the numbers followed two general types, as seen in the examples quoted below :

TABLE I.

	0-100m.	1-200	2-300	3-400	4-500	5-600	6-700	7-800	8-900	9-1000	10-1080
<i>Ranunculus</i> . .	—	2	3	5	7	11	12	18	18	10	2
<i>Drimys</i> . . .	2	2	2	2	2	3	3	2	1	1	1
<i>Pittosporum</i> .	11	11	11	11	8	7	6	6	5	5	1
<i>Colobanthus</i> .	—	—	—	—	—	2	3	3	4	2	—
<i>Coprosma</i> . .	12	12	15	16	17	18	18	16	15	12	3
<i>Metrosideros</i> .	8	8	8	8	5	6	6	2	1	1	1
<i>Ligusticum</i> .	—	1	1	1	2	7	8	9	7	6	3
<i>Veronica</i> . .	6	6	10	14	15	39	41	43	38	26	2
<i>Utricularia</i> .	3	3	3	1	1	1	1	1	—	—	—
<i>Pimelea</i> . . .	4	4	5	5	7	8	8	6	5	4	1

In all cases the figures show a maximum with a regular falling off, but in some, e.g. *Pittosporum*, the maximum is to the north, with no falling off to northwards, in others, e.g. *Ranunculus*, it is further south, with a falling off in both directions, and, as the examples chosen illustrate, the maximum may be at any zone from 5-600 to 8-900 miles. This phenomenon of a simple curve to a maximum (or two) is shown by every genus in the flora.

These curves represent simply the naked unvarnished facts of taxonomic distribution, and they may be considered without reference to my hypothesis of age and area. It is quite clear from them that, for example, the previous distributional history of *Pittosporum* was different from that of *Ranunculus* or *Veronica*. And it is equally clear that in broad outline—which is all that we are as yet concerned with in taxonomic distribution—that distribution was not determined by biological agents, but by a more purely mechanical cause.

Not only so, but it is also extremely difficult to believe that such regularity as this, and regularity of *two* types, would be shown as the result of casual arrivals across a wide expanse of water. It is hard to believe that it can be explained—again in broad outline, and to the extent of say 90 per cent. of the flora—by anything but a previous *land* connexion of New Zealand with Indo-Malaya, and with other sources of flora. My critics, who frequently insist upon applying age and area to individual cases, force upon me the opinion that neither biological agencies nor casual distribution across the sea have had any hand in the present distribution of the New Zealand flora.

This, however, is not my own opinion; what I maintain is that in about 90 per cent. or perhaps more of the cases, the distribution has been by land, and has been chiefly mechanical, so far as its broad outline is concerned. Age and area must not be applied to individual cases.

Another point whose misunderstanding is frequently a stumbling-block in the way of acceptance of age and area, is a confusion of the two types of distribution known as taxonomic and ecological. The former, with which alone age and area is concerned, takes no account of the density or rarity of a given species upon the ground, but simply of the total area over which it is found, while the latter is much more concerned with the density or rarity of a given species under a given set of ecological conditions. From the taxonomic or age and area point of view there is no difference between the species represented by the two groupings of letters below:



and in this connexion it is a pleasure to acknowledge the justice and value of Mrs. Arber's criticism (1), while drawing attention to the fact that she incidentally refers to, that people have in general regarded species with small areas as species that are dying out (cf. 10, p. 349). If this can no longer be accepted, then my general contention that endemics of small area are not necessarily felices of past floras, but are usually young species that have not had time to spread, receives very strong support.

Examining these zonation figures in detail, one at once finds that a great many genera show their maximum at the far north of New Zealand, like *Pittosporum*, *Metrosideros*, and *Utricularia* in Table I. They do not show any falling off to the northwards, even though the figures may be the same for the first two, three, or even four zones. Now a consideration of Diagram I, given on p. 472, will show that the maximum of endemics must *in general* occur somewhere near to the point or zone of original entry of the first species to arrive from abroad. Consequently when one deals, as is now the case, with a large number of different genera, it is clear that the variations to one side or the other will cancel one another, and, therefore, that the region of entry into New Zealand (*as now limited* by the ocean) was within the first 300 or at most 400 miles of the northern part of the North

Island. These species with northern entry are fairly easily segregated from the rest of the flora.

As already indicated in (10), p. 356, Table I, we may look upon about thirty-three families of the ninety-one of the New Zealand flora as showing this northern maximum. But it would be a great mistake to assume that all members of these families entered from the north. So many of them show a northern maximum that their figures swamp those of any that may have entered in any other way, but a more satisfactory result is obtained by going through the figures genus by genus. Even then one finds genera in which there are some wides with evident northern entry, and others with equally evident entry by some other route, but these are few in comparison with the total, and for the present it is better not to attempt to go into too minute detail.

Examining the whole of the genera of the New Zealand flora as given by Cheeseman (all the larger of them are given in Tables V and VI of (9)), I have arrived at the general tentative result given in Table II below. While the northern types seem to divide into a northern (proper) and a Kermadec invasion (11, p. 279), the rest seem to me to split into a western and a southern invasion, a considerable number (western) having their centre of greatest density between 4-500 and 6-700 miles from North Cape; for instance, *Drimys*, *Coprosma*, and *Pimelea* in Table I. In such cases

the very marked tapering off of the numbers both to north and to south makes it fairly probable that they must have commenced at the middle, but these genera run imperceptibly into those of the more southern group which we have already considered in a previous paper, so as to render it very difficult in many cases to separate them. A glance at the map, showing the broadly triangular way in which the water of less than 1,000 fathoms meets the west coast of New Zealand, will help to make it more clear why this should be so. But I am inclined to think, none the less, that my figures indicate that there were probably two different invasions, a western and a southern. At the time when the greater part of the sea floor to 1,000 fathoms was dry land, it is clear that a species arriving at the point where stands the N of 'North I.' would be able to spread out fanwise, and might ultimately reach (the existing) New Zealand along a wide front.

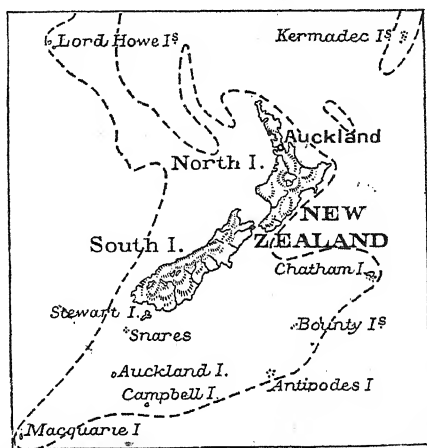


DIAGRAM 2. New Zealand and outlying islands. The dotted line is the 1,000 fathom limit.

There is so distinct a group with maxima in the far south, comprising a great part of eighteen families (10, p. 359), that I am inclined to think that they formed a separate southern invasion, but as I have pointed out (10, 361), the origin of this invasion is a complex question that must be left for the present unanswered, though I am inclined to put it down to Antarctica. It is conceivable, though improbable, that its route was along the southern side of the bridge by which the western invasion probably arrived.

There follows a complete list of the New Zealand genera, giving in each case the invasion to which I incline to attribute it, whether northern (N), northern by the Kermadecs (K), western (W), southern (S), and giving also the northern islands, Kermadecs (K), Norfolk (N), or Lord Howe (H), on which they occur.

TABLE II.

The first entry after the name of a genus, e.g. SW, refers to the invasion or invasions (Northern, Kermadec, Western, or Southern) to which the general distribution of its endemics (and wides when they do not range the whole length of New Zealand) points.

A ? after a letter refers only to that letter, e.g. SW ? means pretty certainly S and perhaps W.

The second entry (K, N, H) refers to the islands (Kermadecs, Norfolk, Howe) in which the genus is found.

Genera in brackets (in small letters) occur only in the Kermadecs in the New Zealand area.

Genera in capitals are endemic ; if in brackets endemic in the Chathams or Auckland.

DICOTYLEDONS	Invasion.	Islands.	DICOTYLEDONS	Invasion.	Islands.
1. Ranunculaceae			27. <i>HOHERIA</i>		
1. <i>Clematis</i>	W	NH	28. <i>Gaya</i>	S	—
2. <i>Myosurus</i>	W?	—	29. <i>Hibiscus</i>	N	NH
3. <i>Ranunculus</i>	SW?	N	11. <i>Tiliaceae</i>		
4. <i>Caltha</i>	WS	—	30. <i>ENTELEA</i>		
2. Magnoliaceae			31. <i>Aristolelia</i>	W?	—
5. <i>Drimys</i>	WN	H	32. <i>Elaeocarpus</i>	W?	—
3. Cruciferae			12. <i>Linaceae</i>		
6. <i>Nasturtium</i>	W?	N	33. <i>Linum</i>	W?	N
7. <i>Cardamine</i>	S	K	13. Geraniaceae		
8. <i>Sisymbrium</i>	W	—	34. <i>Geranium</i>	W?	KN
9. <i>PACHYCLADON</i>			35. <i>Pelargonium</i>	W?	NH
10. <i>Capsella</i>	?	—	36. <i>Oxalis</i>	W?	KH
11. <i>Lepidium</i>	SW	H	14. <i>Rutaceae</i>		
12. <i>NOTOTHLASPI</i>			37. <i>Phedanthum</i>	N	—
4. Violaceae			38. <i>Melicope</i>	N	KH
13. <i>Viola</i>	WS	N	15. <i>Meliaceae</i>		
14. <i>Melicytus</i>	WN	KN	39. <i>Dysoxylum</i>	N	NH
15. <i>Hymenanthera</i>	W	NH	16. <i>Olcaceae</i>		
5. Pittosporaceae			40. <i>Pennantia</i>	NW	N
16. <i>Pittosporum</i>	N	KNH	17. <i>Stackhouseiaceae</i>		
6. Caryophyllaceae			41. <i>Stackhousia</i>	W	—
17. <i>Gypsophila</i>	W?	—	18. <i>Rhamnaceae</i>		
18. <i>Stellaria</i>	SW	—	42. <i>Pomaderris</i>	N	—
19. <i>Colobanthus</i>	S	—	43. <i>Discaria</i>	W?	—
20. <i>Spergularia</i>	?	—	19. <i>Sapindaceae</i>		
7. Portulacaceae			44. <i>Dodonaea</i>	N	NH
21. <i>Claytonia</i>	S	—	45. <i>Alectryon</i>	N	—
22. <i>Montia</i>	S	—	20. <i>Anacardiaceae</i>		
23. <i>HECTORELLA</i>			46. <i>Corynocarpus</i>	KW	K
8. <i>Elatinaceae</i>			21. <i>Coriariaceae</i>		
24. <i>Elatine</i>	?	—	47. <i>Coriaria</i>	WN	K
9. <i>Hypericaceae</i>			22. <i>Leguminosae</i>		
25. <i>Hypericum</i>	W?	—	48. <i>CORALLOSPARTIUM</i>		
10. <i>Malvaceae</i>			49. <i>Carmichaelia</i>	WS	H
26. <i>Plagianthus</i>	W?	—	50. <i>NOTOSPARTIUM</i>		

DICOTYLEDONS			DICOTYLEDONS		
Invasion.	Islands.		Invasion.	Islands.	
51. <i>Clanthus</i>	N	N	103. <i>Galium</i>	W?	—
52. <i>Swainsona</i>	W	—	104. <i>Asperula</i>	W?	—
53. ( <i>Canavalia</i> )	—	(KNH)	38. Compositae	—	—
54. <i>Sophora</i>	W?	H	105. ( <i>Ageratum</i> )	—	(K)
23. Rosaceae	—	—	106. <i>Lagenophora</i>	W?	K
55. <i>Rubus</i>	W?	—	107. <i>Brachycome</i>	SW	H
56. <i>Geum</i>	W? S?	—	108. <i>Olearia</i>	W	H
57. <i>Potentilla</i>	W?	—	109. (PLEUROPHYLLUM)	—	—
58. <i>Acaena</i>	?	K	110. <i>Celmisia</i>	S?	—
24. Saxifragaceae	—	—	111. <i>Vittadinia</i>	W?	—
59. <i>Donatia</i>	S	—	112. HAASTIA	—	—
60. <i>Quintinia</i>	WN?	—	113. <i>Gnaphalium</i>	WS?	KNH
61. I.XERBA	—	—	114. <i>Raoulia</i>	S?	—
62. CARPODETUS	—	—	115. <i>Helichrysum</i>	W	—
63. <i>Ackama</i>	N	—	116. <i>Cassinia</i>	W	H
64. <i>Weinmannia</i>	W?	—	117. <i>Craspedia</i>	W?	—
25. Crassulaceae	—	—	118. <i>Siegesbeckia</i>	K	K
65. <i>Tylosa</i>	WS?	—	119. <i>Bidens</i>	K	KNH
26. Droseraceae	—	—	120. <i>Cotula</i>	SW	KNH
66. <i>Drosera</i>	NWS?	—	121. <i>Centipeda</i>	W?	—
27. Haloragidaceae	—	—	122. <i>Abrotanella</i>	S	—
67. <i>Haloragis</i>	?	K	123. <i>Erechtites</i>	WS	NH
68. <i>Myriophyllum</i>	?	—	124. BRACHYGLOTTIS	—	—
69. <i>Gunnera</i>	S?	—	125. <i>Senecio</i>	W	KNH
70. <i>Callitriche</i>	?	K	126. <i>Microseris</i>	W?	—
28. Myrtaceae	—	—	127. <i>Picris</i>	N?	N
71. <i>Leptospermum</i>	NW	H	128. <i>Crepis</i>	S?	—
72. <i>Metrosideros</i>	N	KH	129. <i>Taraxacum</i>	W?	—
73. <i>Myrtus</i>	N	—	130. <i>Sonchus</i>	W?	KNH
74. <i>Eugenia</i>	N	—	39. Styliidiaceae	—	—
29. Onagraceae	—	—	131. <i>Phyllachne</i>	S	—
75. <i>Epilobium</i>	SW?	—	132. OKEOSTYLIDIUM	—	—
76. <i>Fuchsia</i>	?	—	133. <i>Forstera</i>	S?	—
30. Passifloraceae	—	—	40. Goodeniaceae	—	—
77. <i>Passiflora</i>	N	NH	134. <i>Selliera</i>	W?	—
31. Cucurbitaceae	—	—	135. ( <i>Scaevola</i> )	—	(K)
78. <i>Sicyos</i>	KN	KNH	41. Campanulaceae	—	—
32. Ficoidene	—	—	136. COLENSOA	—	—
79. <i>Mesembryanthemum</i>	NW	KNH	137. <i>Pratia</i>	WS?	—
80. <i>Tetragonia</i>	NW	KNH	138. <i>Lobelia</i>	WS?	KNH
33. Umbelliferae	—	—	139. <i>Isotoma</i>	SW?	—
81. <i>Hydrocotyle</i>	W	KH	140. <i>Wahlenbergia</i>	W?	KNH
82. <i>Asorella</i>	S?	—	42. Ericaceae	—	—
83. <i>Eryngium</i>	W	—	141. <i>Gaultheria</i>	W	—
84. <i>Actinotus</i>	S	—	142. <i>Pernettya</i>	S	—
85. <i>Apium</i>	N?	KNH	43. Epacridaceae	—	—
86. <i>Oreomyrrhis</i>	W	—	143. <i>Pentachondra</i>	W?	—
87. <i>Crantzia</i>	W?	—	144. <i>Cyathodes</i>	SW	—
88. <i>Aciphylla</i>	SW?	—	145. <i>Leucopogon</i>	NW?	H
89. <i>Ligusticum</i>	S	—	146. <i>Epacris</i>	W?	—
90. <i>Angelica</i>	S?	—	147. <i>Archeria</i>	W?	—
91. <i>Daucus</i>	W?	—	148. <i>Dracophyllum</i>	W	H
34. Araliaceae	—	—	44. Primulaceae	—	—
92. STILBOCARPA	—	—	149. <i>Samolus</i>	?	KN
93. <i>Aralia</i>	S	—	45. Myrsinaceae	—	—
94. <i>Panax</i>	W	KH	150. <i>Myrsine</i> (incl. <i>Rapanea</i> )	W?	KNH
95. <i>Meryta</i>	N	N	46. Sapotaceae	—	—
96. <i>Schefflera</i>	?	—	151. <i>Sideroxylon</i>	N	NH
97. PSEUDOPANAX	—	—	47. Oleaceae	—	—
35. Cornaceae	—	—	152. <i>Olea</i>	N	NH
98. COROKIA	—	—	48. Apocynaceae	—	—
99. <i>Griselinia</i>	W?	—	153. <i>Parsonsia</i>	W?	—
36. Caprifoliaceae	—	—	49. Loganiaceae	—	—
100. <i>Alseuosmia</i>	N	—	154. <i>Mitrasacme</i>	?	—
37. Rubiaceae	—	—	155. <i>Logania</i>	?	—
101. <i>Coprosma</i>	W	KNH	156. <i>Gentostoma</i>	N	H
102. <i>Nertera</i>	NWS?	—			

DICOTYLEDONS			DICOTYLEDONS		
	<i>Invasion.</i>	<i>Islands.</i>		<i>Invasion.</i>	<i>Islands.</i>
50. Gentianaceae			205. <i>Laurelia</i>	N?	—
157. <i>Sebaca</i>	W?	—	69. Lauraceae		
158. <i>Gentiana</i>	WS	—	206. <i>Beilschmiedia</i>	N	—
159. <i>Liparophyllum</i>	S?	—	207. <i>Litsaea</i>	N	—
51. Boraginaceae			208. <i>Cassytha</i>	N	—
160. <i>Myosotis</i>	WS	—	70. Proteaceae		
161. ( <i>MYOSOTIDIUM</i> )			209. <i>Persoonia</i>	N	—
162. <i>TETRACHONDRA</i>			210. <i>Knightia</i>	N	—
52. Convolvulaceae			71. Thymelaeaceae		
163. <i>Ipomoea</i>	?	KNH	211. <i>Pimelea</i>	W	H
164. <i>Calystegia</i>	WN?	KNH	212. <i>Drapetes</i>	S?	—
165. <i>Convolvulus</i>	W?	—	72. Lorantheae		
166. <i>Dichondra</i>	W?	—	213. <i>Loranthus</i>	WS	—
167. <i>Cuscuta</i>	?	—	214. <i>TUPEIA</i>		
53. Solanaceae			215. <i>Viscum</i>	WS	NH
168. <i>Solanum</i>	W?	KNH	73. Santalaceae		
54. Scrophulariaceae			216. <i>Fusanus</i>	N	—
169. <i>Calceolaria</i>	W?	—	217. <i>Exocarpus</i>	WS	NH
170. <i>Mimulus</i>	W?	—	74. Balanophoraceae		
171. <i>Macus</i>	W?	—	218. <i>DACTYLANTHUS</i>		
172. <i>Gratiola</i>	W?	—	75. Euphorbiaceae		
173. <i>Glossostigma</i>	W?	—	219. <i>Euphorbia</i>	?	NH
174. <i>Limosella</i>	W?	—	220. <i>Poranthera</i>	W?	—
175. <i>Veronica</i>	S	KN	221. ( <i>Aleurites</i> )		(K)
176. <i>Ourisia</i>	S	—	222. ( <i>Homalanthus</i> )		(K)
177. <i>Euphrasia</i>	WS?	—	76. Urticaceae		
178. <i>ANAGOSPERMA</i>			223. <i>Paratrophis</i>	N?	—
179. <i>SIPHONIDIUM</i>			224. <i>Urtica</i>	SW?	—
55. Lentibulariaceae			225. <i>Elatostema</i>	N	H
180. <i>Utricularia</i>	NWS?	—	226. ( <i>Boehmeria</i> )		(KNH)
56. Gesneriaceae			227. <i>Parietaria</i>	N	KNH
181. <i>RHABDOTHAMNUS</i>			228. <i>Australina</i>	W?	—
57. Myoporaceae			77. Cupuliferae		
182. <i>Myoporum</i>	NW	KNH	229. <i>Fagus</i>	W?	—
58. Verbenaceae					
183. <i>Vitex</i>	N	N	MONOCOTYLEDONS		
184. <i>TEUCRIDIMUM</i>			78. Orchidaceae		
185. <i>Avicennia</i>	N	H	230. <i>Dendrobium</i>	?	NH
59. Labiatae			231. <i>Bulbophyllum</i>	N	NH
186. <i>Mentha</i>	W?	—	232. <i>Earina</i>	N?	—
187. <i>Scutellaria</i>	W	—	233. <i>Sarcochilus</i>	N?	—
60. Plantaginaceae			234. <i>Spiranthes</i>	N	—
188. <i>Plantago</i>	SW	H	235. <i>Thelymitra</i>	N	—
61. Nyctaginaceae			236. <i>Orthoceras</i>	N	—
189. <i>Pisonia</i>	N	NH	237. <i>Microtis</i>	N?	KNH
62. Illecebraceae			238. <i>Prasophyllum</i>	?	—
190. <i>Scleranthus</i>	W?	—	239. <i>Calcana</i>	N	—
63. Amarantaceae			240. <i>Pterostylis</i>	W	—
191. <i>Alternanthera</i>	N	—	241. <i>Acianthus</i>	K	K
64. Chenopodiaceae			242. <i>Cyrtostylis</i>	N	—
192. <i>Rhagodia</i>	K	KH	243. <i>Calochilus</i>	N?	—
193. <i>Chenopodium</i>	WS?	—	244. <i>Lyperanthus</i>	S?	—
194. <i>Atriplex</i>	W?	H	245. <i>Caladenia</i>	WS?	—
195. <i>Salicornia</i>	W	H	246. <i>Chiloglottis</i>	W?	—
196. <i>Suaeda</i>	W	—	247. <i>Adenochilus</i>	W?	—
197. <i>Salsola</i>	W	—	248. <i>TOWNSONIA</i>		
65. Polygonaceae			249. <i>Corysanthes</i>	N?	—
198. <i>Polygonum</i>	W?	—	250. <i>Gastrodia</i>	NW	—
199. <i>Rumex</i>	?	KNH	79. Iridaceae		
200. <i>Muehlenbeckia</i>	W?	NH	251. <i>Libertia</i>	W?	—
66. Piperaceae			80. Amaryllidaceae		
201. <i>Piper</i>	K	KNH	252. <i>Hypoxis</i>	W?	—
202. <i>Peperomia</i>	K	KNH	81. Liliaceae		
67. Chloranthaceae			253. <i>Rhipogonum</i>	W?	N
203. <i>Ascarina</i>	?	K	254. <i>Enargea</i>	W?	—
68. Monimiaceae			255. <i>Cordylone</i>	N?	KN
204. <i>Hedycarya</i>	N?	—	256. <i>Astelia</i>	N	—

MONOCOTYLEDONS	Invasion.	Islands.	MONOCOTYLEDONS	Invasion.	Islands.
257. <i>Dianella</i>	N?	NH	290. <i>Schoenus</i>	WN	—
258. <i>Phormium</i>	?	N	291. <i>Cladium</i>	N	H
259. <i>Bulbinella</i>	?	—	292. <i>Lepidosperma</i>	N	—
260. <i>Arthropodium</i>	?	—	293. <i>Gahnia</i>	WN	H
261. <i>Herpotion</i>	W?	—	294. <i>Oreoholus</i>	?	—
262. <i>Iphigenia</i>	S?	—	295. <i>Uncinia</i>	SW	H
82. Juncaceae			296. <i>Carex</i>	SW	KNH
263. <i>Rostkovia</i>	S	—	91. Gramineae		
264. <i>Juncus</i>	NWS	H	297. <i>Imperata</i>	N	K
265. <i>Luzula</i>	S	H	298. <i>Zoysia</i>	N	—
83. Palmae			299. <i>Paspalum</i>	N	NH
266. <i>Rhopalostylis</i>	N	KN	300. <i>Isachne</i>	N	—
84. Pandanaceae			301. ( <i>Panicum</i> )		(KN)
267. <i>Freyinetia</i>	N	N	302. <i>Oplismenus</i>	K	KNH
85. Typhaceae			303. ( <i>Cenchrus</i> )		(K)
268. <i>Typha</i>	N	KN	304. <i>Spinifex</i>	N	H
269. <i>Sparganium</i>	N	—	305. <i>Ehrharta</i>	S?	—
86. Lemnaceae			306. <i>Microlaena</i>	?	N
270. <i>Lemna</i>	?	—	307. <i>Hierochloa</i>	?	—
87. Naiadaceae			308. <i>Stipa</i>	?	—
271. <i>Triglochin</i>	W?	—	309. <i>Echinopogon</i>	NW?	NH
272. <i>Potamogeton</i>	W?	—	310. <i>Alopecurus</i>	W?	—
273. <i>Ruppia</i>	W?	—	311. <i>Sporobolus</i>	N?	N
274. <i>Zannichellia</i>	W	—	312. <i>SIMPLICIA</i>		
275. <i>Lepilaena</i>	?	—	313. <i>Agrostis</i>	SW	—
276. <i>Zostera</i>	?	—	314. <i>Deyeuxia</i> ( <i>Calama-</i>		
88. Centrolepidaceae			<i>grostis</i> )	?	KNH
277. <i>Trithuria</i>	?	—	315. <i>Dichelachne</i>	NW	NH
278. <i>Centrolepis</i>	?	—	316. <i>Deschampsia</i>	SW	—
279. <i>Gaimardia</i>	S	—	317. <i>Trisetum</i>	NS?	—
89. Restionaceae			318. <i>Amphibromus</i>	N	—
280. <i>Lepyrodia</i>	?	—	319. <i>Danthonia</i>	S	—
281. <i>Leptocarpus</i>	?	—	320. ( <i>Eleusine</i> )		(K)
282. <i>Hypolaena</i>	?	—	321. <i>Arundo</i>	W?	—
90. Cyperaceae			322. <i>Triodia</i>	S	—
283. <i>Kyllinga</i>	N	NH	323. <i>Koeleria</i>	S	—
284. <i>Cyperus</i>	N	N	324. <i>Poa</i>	WS	KNH
285. <i>Mariscus</i>	N	NH	325. <i>Atropis</i>	W?	—
286. <i>Eleocharis</i>	NW?	N	326. <i>Festuca</i>	W?	—
287. <i>Fimbristylis</i>	N	—	327. <i>Bromus</i>	N	—
288. <i>Scirpus</i>	NW?	KNH	328. <i>Agropyrum</i>	NW?	KNH
289. <i>Carpha</i>	WS	—	329. <i>Asperella</i>	?	—

Looking at the map, it is evident that the western invasion most probably arrived by the ridge which reaches the western coast of New Zealand, and has two branches, one by Lord Howe Island, and one by Norfolk (the new map is a more recent one than the old, which shows the ridge not quite reaching Norfolk). The northern invasion is not so certain as to route. It may have passed by way of the Kermadecs, though we have already seen (11, p. 280) that this seems improbable; or it may have come by way of the belt of comparatively shallow water which unites the northern end of New Zealand with New Caledonia, and which passes through Norfolk Island. As this water is deeper than that over the western ridge, the northern may have been a more ancient invasion than the western, and there are other facts which point to the possibility of this, as we shall see in later papers. But in any case, northern, western, and Kermadec invasions all came from parts of



Indo-Malaya, and there now exist on their probable tracks the islands of Lord Howe, Norfolk, and the Kermadecs.

One may go on to say at once, that while these islands show great traces of all these invasions, they do not by any means, even if all their floras be added together, contain the whole of the genera, or even of the wides, of those invasions. In actual fact, of the genera in the above list that

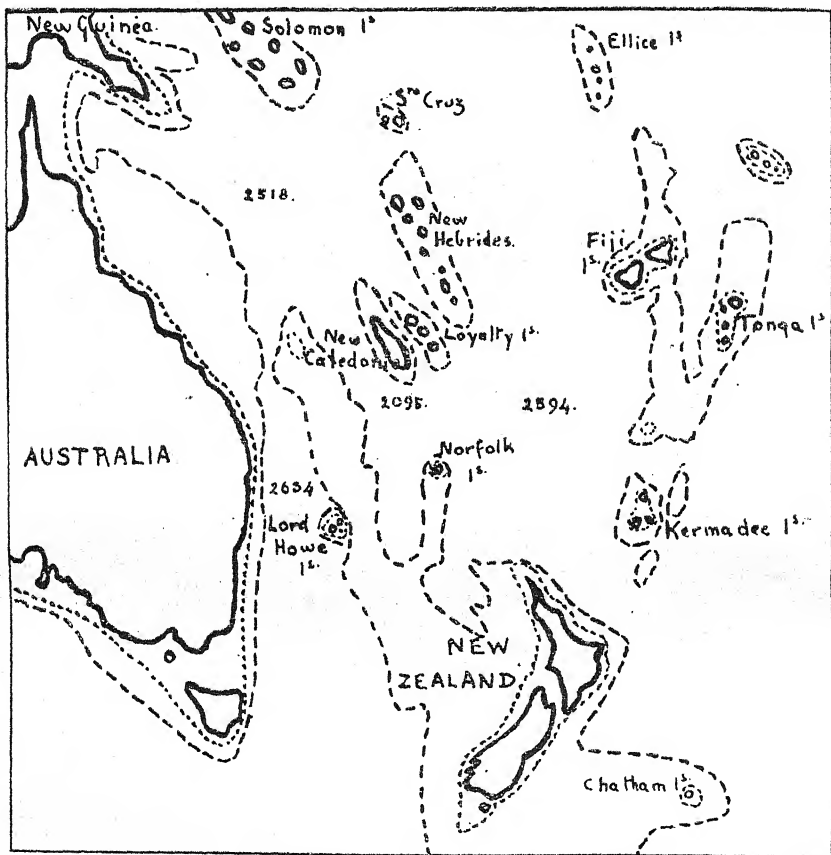


DIAGRAM 3. Soundings in the New Zealand area. ----- 100 fathoms. ----- 1,000 fathoms. Numbers inserted here and there give the depth in fathoms at those points.

are marked N, W, K, NW, NW?, NWS, NWS?, N?, NS?, WN, WN?, WS, WS?, W?S?, KW, KN, or SW, they contain 82 out of 152, or 54 per cent., and if the genera marked W? be added they contain 96 out of 219, or 44 per cent. Of the New Zealand wides of these genera 50 (38 per cent.) occur in the islands (29 Dicotyledons and 21 Monocotyledons) and 81 do not (33 and 48). One may feel inclined to say that this shows that transport must have been casual, but one must remember the very small area of these islands. Lord Howe is 7 miles long and has a maximum

breadth of 1 mile, and Norfolk is only 5 miles long and a little broader, while the largest of the Kermadecs has an area of 12 sq. miles, and in all three cases the soil is purely volcanic. It would not, I think, be difficult to pick out of New Zealand, even in the centre, which is the richest area, a small area of uniform soil which would not contain more species than one of these islands. As a test, it is difficult to find an area of this size that has been sufficiently well botanized, but in the Flora of Cambridgeshire by Babington, I find that of 388 genera with 950 species in the county, only 230 genera with 388 species occur in the Wisbech district, which is about 15 miles by 10 with fairly uniform soil and conditions, though even there a number of coast (*halophytic*) species enter in one part. I do not, therefore, feel surprised that these little islands do not contain more of the northern and western invasions of New Zealand. It is worthy of a passing note that Lord Howe contains 160 species (52 locally endemic) and Norfolk 162 (43 locally endemic), and that these islands are of approximately the same area.

After these preliminary remarks, we may go on to the usual method of prediction and verification, and endeavour to see what can be made of the floras of these islands in this manner. For convenience in making predictions, we give below the complete list of the genera of these islands, with the islands (K, N, or H) upon which they occur. The list is compiled from the Floras of Howe by Hemsley (3) and Oliver (5), Norfolk by Maiden (4), and the Kermadecs by Cheeseman (2).

TABLE III.

## FLORA OF LORD HOWE, NORFOLK, AND THE KERMADEC ISLANDS.

Families and genera given in italics are found in New Zealand.  
Genera in capitals are endemic.

	No. of spp.	Occur in		No. of spp.	Occur in
1. <i>Ranunculaceae</i>			8. <i>Pittosporaceae</i>		
1. <i>Clematis</i>	1	NH	14. <i>Pittosporum</i>	3	KNH
2. <i>Ranunculus</i>	1	N	9. <i>Frankeniaceae</i>		
2. <i>Magnoliaceae</i>			15. <i>Frankenia</i>	1	N
3. <i>Drimys</i>	1	H	10. <i>Malvaceae</i>		
3. <i>Menispermaceae</i>			16. <i>Malvastrum</i>	1	N
4. <i>Stephania</i>	1	H	17. <i>Abutilon</i>	1	N
4. <i>Cruciferae</i>			18. <i>Hibiscus</i>	3	NH
5. <i>Nasturtium</i>	1	N	19. <i>Lagunaria</i>	1	NH
6. <i>Cardamine</i>	1	K	11. <i>Sterculiaceae</i>		
7. <i>Cakile</i>	1	N	20. <i>Ungeria</i>	1	N
8. <i>Lepidium</i>	1	H	12. <i>Linaceae</i>		
5. <i>Capparidaceae</i>			21. <i>Linum</i>	1	N
9. <i>Capparis</i>	1	N	13. <i>Geraniaceae</i>		
6. <i>Violaceae</i>			22. <i>Geranium</i>	2	KN
10. <i>Viola</i>	1	N	23. <i>Pelargonium</i>	1	NH
11. <i>Melicytus</i>	1	KN	24. <i>Oxalis</i>	1	KH
12. <i>Hymenantihera</i>	2	NH	14. <i>Rutaceae</i>		
7. <i>Bixineae</i>			25. <i>Melicope</i>	2	KH
13. <i>Xylosma</i>	1	H	26. <i>Evodia</i>	2	NH

	No. of spp.	Occur in		No. of spp.	Occur in
27. <i>Acronychia</i>	2	NH	75. <i>Siegesbeckia</i>	1	K
28. <i>Xanthoxylum</i>	2	NH	76. <i>Wedelia</i>	1	NH
15. <i>Meliaceae</i>			77. <i>Cassinia</i>	1	H
29. <i>Dysoxylum</i>	2	NH	78. <i>Bidens</i>	1	KNH
16. <i>Oleaceae</i>			79. <i>Erechtites</i>	2	NH
30. <i>Pennantia</i>	1	N	80. <i>Cotula</i>	1	KNH
17. <i>Celastraceae</i>			81. <i>Senecio</i>	2	KNH
31. <i>Elaeodendron</i>	1	NH	82. <i>Sonchus</i>	1	KNH
18. <i>Sapindaceae</i>			83. <i>Picris</i>	1	N
32. <i>Guioa</i>	1	H	34. <i>Goodeniaceae</i>		
33. <i>Dodonaea</i>	1	NH	84. <i>Scaevola</i>	2	KH
19. <i>Anacardiaceae</i>			35. <i>Campanulaceae</i>		
34. <i>Corynocarpus</i>	1	K	85. <i>Lobelia</i>	1	KNH
20. <i>Coriariaceae</i>			86. <i>Wahlenbergia</i>	1	KNH
35. <i>Coriaria</i>	1	K	36. <i>Epacridaceae</i>		
21. <i>Leguminosae</i>			87. <i>Leucopogon</i>	1	H
36. <i>Millettia</i>	1	N	88. <i>Dracophyllum</i>	1	H
37. <i>Glycine</i>	1	N	37. <i>Plumbaginaceae</i>		
38. <i>Carmichaelia</i>	1	H	89. <i>Plumbago</i>	1	N
39. <i>Mucuna</i>	1	H	38. <i>Primulaceae</i>		
40. <i>Canavalia</i>	1	KNH	90. <i>Samolus</i>	1	KN
41. <i>Vigna</i>	1	NH	39. <i>Myrsinaceae</i>		
42. <i>Sophora</i>	1	H	91. <i>Rapanea</i>	4	KNH
43. <i>Caesalpinia</i>	1	NH	92. <i>Aegiceras</i>	1	H
44. <i>Clanthus</i>	1	N	40. <i>Sapotaceae</i>		
22. <i>Rosaceae</i>			93. <i>Sideroxylon</i>	2	NH
45. <i>Acacia</i>	1	K	41. <i>Styracaceae</i>		
23. <i>Saxifragaceae</i>			94. <i>Symplocos</i>	1	H
46. <i>COLMEIROA</i>	1	H	42. <i>Oleaceae</i>		
24. <i>Haloragidaceae</i>			95. <i>Jasminum</i>	2	NH
47. <i>Haloragis</i>	1	K	96. <i>Olea</i>	2	NH
48. <i>Callitriche</i>	1	K	97. <i>Notelea</i>	1	H
25. <i>Myrtaceae</i>			43. <i>Apocynaceae</i>		
49. <i>Metrosideros</i>	2	KH	98. <i>Melodinus</i>	1	N
50. <i>Leptospermum</i>	1	H	99. <i>Alyxia</i>	4	NH
51. <i>Melaleuca</i>	1	H	100. <i>Ochrosia</i>	1	H
52. <i>Acicalyptus</i>	1	H	101. <i>Lyonsia</i>	1	H
53. <i>Rhodomyrtus</i>	1	N	44. <i>Asclepiadaceae</i>		
26. <i>Lythraceae</i>			102. <i>Vincetoxicum</i>	1	H
54. <i>Lythrum</i>	1	N	103. <i>Tylophora</i>	1	NH
27. <i>Passifloraceae</i>			104. <i>Marsdenia</i>	1	H
55. <i>Passiflora</i>	3	NH	45. <i>Loganiaceae</i>		
28. <i>Cucurbitaceae</i>			105. <i>Geniostoma</i>	1	H
56. <i>Bryonopsis</i>	1	N	46. <i>Gentianaceae</i>		
57. <i>Sicyos</i>	1	KNH	106. <i>Erythraea</i>	1	N
58. <i>Melothria</i>	1	N	47. <i>Boraginaceae</i>		
29. <i>Ficoideae</i>			107. <i>Cynoglossum</i>	1	N
59. <i>Mesembryanthemum</i>	2	KNH	48. <i>Convolvulaceae</i>		
60. <i>Tetragonia</i>	2	KNH	108. <i>Ipomoea</i>	5	KNH
61. <i>Sesuvium</i>	1	H	109. <i>Calystegia</i>	4	KNH
30. <i>Umbelliferae</i>			49. <i>Solanaceae</i>		
62. <i>Hydrocotyle</i>	2	KH	110. <i>Solanum</i>	3	KNH
63. <i>Apium</i>	2	KNH	50. <i>Scrophulariaceae</i>		
31. <i>Araliaceae</i>			111. <i>Veronica</i>	2	KN
64. <i>Nothopanax</i>	2	KH	51. <i>Gesneriaceae</i>		
65. <i>Meryia</i>	2	N	112. <i>NEGRIA</i>	1	H
32. <i>Rubiaceae</i>			52. <i>Bignoniaceae</i>		
66. <i>Randia</i>	1	H	113. <i>Tecoma</i>	2	NH
67. <i>Psychotria</i>	1	H	53. <i>Acanthaceae</i>		
68. <i>Coprosma</i>	8	KNH	114. <i>Eranthemum</i>	1	H
33. <i>Compositae</i>			54. <i>Myoporaceae</i>		
69. <i>Ageratum</i>	1	K	115. <i>Myoporum</i>	3	KNH
70. <i>Lagenophora</i>	1	K	55. <i>Verbenaceae</i>		
71. <i>Vernonia</i>	1	N	116. <i>Verbena</i>	1	N
72. <i>Brachycome</i>	1	H	117. <i>Vitex</i>	1	N
73. <i>Olearia</i>	2	H	118. <i>Avicennia</i>	1	H
74. <i>Gnaphalium</i>	3	KNH			

		No. of spp.	Occur in			No. of spp.	Occur in
56. Labiatae				73. Amaryllidaceae			
119. Westringia	1	H		161. Crinum	1	N	
120. Plectranthus	1	H		74. Liliaceae			
57. Plantaginaceae				162. Smilax	1	? NH	
121. Plantago	1	H		163. Rhipogonum	1	N	
58. Nyctaginaceae				164. Geitonoplesium	1	NH	
122. Boerhaavia	1	H		165. Cordylina	2	KN	
123. Pisonia	1	NH		166. Phormium	1	N	
59. Amarantaceae				167. Dianella	2	NH	
124. Achyranthes	2	NH		75. Commelinaceae			
60. Chenopodiaceae				168. Commelina	1	NH	
125. Rhagodia	2	KH		76. Flagellariaceae			
126. Atriplex	1	H		169. Flagellaria	1	H	
127. Salicornia	1	H		77. Juncaceae			
61. Polygonaceae				170. Juncus	1	H	
128. Rumex	2	KNH		171. Luzula	1	H	
129. Muehlenbeckia	2	NH		78. Palmae			
62. Piperaceae				172. Rhopalostylis	2	KN	
130. Macropiper	1	KNH		173. HEDYSCEPE	1	H	
131. Peperomia	4	KNH		174. Clinostigma	1	H	
63. Chloranthaceae				175. HOWEA	2	H	
132. Ascarina	1	K		79. Pandanaceae			
64. Lauraceae				176. Pandanus	1	H	
133. Cryptocarya	2	H		177. Freycinetia	1	N	
65. Thymelaeaceae				80. Typhaceae			
134. Pimelea	1	H		178. Typha	1	KN	
135. Wikstroemia	1	N		81. Araceae			
66. Loranthaceae				179. Colocasia	1	N	
136. Korthalsella	1	NH		82. Cyperaceae			
67. Santalaceae				180. Cyperus	2	N	
137. Exocarpos	2	NH		181. Mariscus	1	NH	
68. Euphorbiaceae				182. Kyllinga	1	NH	
138. Aleurites	1	K		183. Cladium	1	H	
139. Homalanthus	1	KH		184. Eleocharis	1	N	
140. Euphorbia	4	NH		185. Gahnia	1	H	
141. Hemicyclia	1	H		186. Uncinia	1	II	
142. Baloghia	1	NH		187. Scirpus	5	KNH	
143. Excoecaria	1	N		188. Carex	5	KNH	
69. Urticaceae				83. Gramineae			
144. Celtis	2	NH		189. Oplismenus	2	KNH	
145. Malaisia	2	NH		190. Spizifex	1	H	
146. Ficus	1	H		191. Paspalum	2	NH	
147. Pseudomorus	1	N		192. Panicum	4	KN	
148. Procris	1	N		193. Imperata	1	K	
149. Elatostema	1	H		194. Cenchrus	1	K	
150. Boehmeria	3	KNH		195. Andropogon	2	N	
151. Parietaria	1	KNH		196. Microlaena	1	N	
70. Hydrocharidaceae				197. Echinopogon	1	NH	
152. Halophila	1	H		198. Sporobolus	1	N	
71. Orchidaceae				199. Calamagrostis	1	KNH	
153. Oberonia	1	N		200. Dichelachne	2	NH	
154. Dendrobium	4	NH		201. Phragmites	1	H	
155. Bulbophyllum	2	NH		202. Cynodon	1	N	
156. Phreatia	1	N		203. Eleusine	1	K	
157. Microtis	1	KNH		204. Poa	2	KH	
158. Acianthus	1	K		205. Agropyrum	1	KNH	
159. Cleisostoma	1	H		206. Triticum	1	N	
72. Iridaceae							
160. Moraea	1	H					

(1) If, as I maintain, these islands lie upon the tracks of previous plant-invasions of New Zealand by *land*, then one will expect to find that a large part of their flora consists of families, genera, and species which also occur in New Zealand.

TABLE IV.

	Species.	of	Genera.	and	Families.
Occur in N.Z.	93 (none) (none) (none)		75 23 17 (none)		41 same 15 (others) 11 (others)
Total occurring in N.Z.	93 sp.		115 gen.		67 fams.
Not found in N.Z.	209 do.		91 do.		16 do.
Total for all the islands	302		206		83

Thus nearly one-third of the species, more than half of the genera, and four-fifths of the families occur in New Zealand.

(2) One will expect the 23 genera of the same families to be chiefly represented by endemic forms, or by Australian or Polynesian wides which have been too late to reach New Zealand. In actual fact the 23 genera are represented in the islands by 31 species, of which 21 are endemic to the islands, 1 (*Imperata Cheesemani*) to the Kermadecs, 8 to Norfolk, 11 to Howe, and 1 (*Mariscus haematodes*) to both Norfolk and Howe. A further 7 species are Australian wides, 2 are Polynesian wides, and 1 (*Nasturtium sylvestre*) is probably introduced. The prophecy is thus fully borne out.

(3) One will also expect the 17 New Zealand genera, belonging to the 15 families which are not represented in New Zealand by any species that occur on these islands, to be similarly made up of endemic species and Australian and Polynesian wides. In actual fact these 17 genera show on the islands 26 species, which are composed of 18 endemics, 7 Australian wides, and 1 Polynesian wide.

(4) The 11 New Zealand families, which are not represented on the islands either by genera or by species that occur in New Zealand, are Saxifragaceae, Goodeniaceae, Apocynaceae, Gentianaceae, Boraginaceae, Gesneriaceae, Labiatae, Amarantaceae, Lauraceae, Iridaceae, and Amaryllidaceae, and are represented on the islands, as one would rather expect, by 11 endemic species, including two endemic genera (*Colmeiropa* and *Negria*) and 10 Australian wides. One of the endemic species, *Moraea Robinsoniana*, belongs to an African genus, which probably arrived by sea carriage.

(5) Passing on now to the species, genera, and families given in Table IV as not found in New Zealand, one will expect that a large proportion of the species will be endemic in the islands. In fact, of the 209 species that occur on the islands and have not been found in New Zealand, no less than 105 appear to be endemic to the islands.

(6) One will expect that the remaining 104 species will be Australian or Polynesian wides, especially the former. In fact 93 of them are Australian and 9 Polynesian, while 2 (*Nasturtium sylvestre* and *Cakile maritima*) are perhaps introduced, being European types.

(7) One will also expect to find that the 209 species that do not reach New Zealand will be largely members of genera and still more of families that do reach it. Those families and genera that reach New Zealand will on the whole be the earliest arrivals of their respective affinity groups. These groups will thus, so to speak, have the start of the others, and there will be more likely to be more of them in the islands than of other groups, of which none have reached New Zealand. Testing this we find in the island flora :

TABLE V.

Not reaching N.Z.	209 species of 155 genera and 74 families
Found in N.Z. of these	(none) 64 " (with 130 spp.) and 58 families
Leaving	91 " " 112 " " 16 "

(8) One will expect to find the families and genera that have reached New Zealand better represented in the islands, on the whole, as being older, than those that have not.

TABLE VI.

The 58 families contain 137 genera, or 2.3 genera per family
" 16 " 18 " 1.1 "
" 64 genera contain 130 species, or 2.0 species per genus
" 91 " 112 " 1.2 "

(9) The 16 families that do not reach New Zealand at all, are all, as one would expect (they being probably young, and therefore on the average small, in the islands), small and not widely distributed in the islands. They are Menispermaceae (1 sp. Howe), Capparidaceae (1 Norf.), Bixaceae (1 Howe), Frankeniaceae (1 Norf.), Sterculiaceae (1 Norf.), Celastraceae (1 Norf., 1 Howe), Lythraceae (1 Norf.), Plumbaginaceae (1 Norf.), Styraceae (1 Howe), Asclepiadaceae (2 on Howe only 1 Norf., 1 Howe), Bignoniaceae (1 Norf., 1 Howe), Acanthaceae (1 Howe), Hydrocharidaceae (1 Howe), Commelinaceae (1 Norf., 1 Howe), Flagellariaceae (1 Howe), Araceae (1 Norf.). All are small, and little distributed among the islands. Similarly, of the 91 genera, 65 have only one species reaching one island, 10 one species reaching two.

(10) Of the 91 genera that do not occur in New Zealand, one will expect the greater proportion (both absolute and per family) to belong to families that have reached New Zealand, for these will be the older families in their affinity circles. In actual fact 73 belong to 42 families that have reached New Zealand, or four-fifths of the total, and 1.7 genera per family, while 18 belong to 16 families that have not reached New Zealand, or one-fifth of the total, and 1.1 genera per family. The prophecy is thus borne out.

(11) To turn now to another type of consideration, one will expect the bulk of the floras of these islands to belong to genera and families that in Table II are marked as probably belonging to northern, Kermadec, or

western invasions of New Zealand. In actual fact, of the 115 genera that reach New Zealand, we find that they are marked as follows :

TABLE VII.

N	28			WN	4				
N?	6			WN?	1				
NW	6	} 46		W	11	} 38	SW	6	
NWS	1			W?	14		SW?	1	} 10
NW?	5			WS?	2		S or S?	3	
				WS	6				
			K	7					
			KN	1	} 9				
			KW	1					
									1 } 12

Thus 55, or just less than half, are of unquestionably northern origin (N or K) as judged simply by their distribution in New Zealand, and 38 more are probably western, while 12 have so few species, and so widely distributed in New Zealand, that one cannot tell from their local distribution by which route they may have entered.

There remain 10 genera, marked in Table II as SW or S. The six SW genera are represented in the islands by (i) *Lepidium Howeiiinsulae*, endemic and allied to *L. oleraceum* of New Zealand, therefore probably derived from New Zealand; (ii) *Brachycome segmentosa*, endemic and allied to an Australian species; (iii) *Cotula australis*, found in all three islands, and in New Zealand down to Foveaux Strait, as well as in Australia, and therefore probably an Australian species which entered by the west; (iv) *Plantago Headleyi* (Howe), endemic and near *P. aucklandica* of New Zealand, and therefore probably derived from New Zealand; (v) *Uncinia filiformis* (Howe), found in New Zealand from 280 miles south of N. Cape down to Stewart Island, and therefore possibly a western type; also found in Australia; and (vi) *Carex semiforsteri* (Kermadecs), found down to Foveaux Strait in New Zealand and thus probably not southern, *C. Neesiana*, endemic in Norfolk, *C. inversa* (Norfolk), down to Foveaux Strait and in Australia, and therefore (on both counts) probably western, *C. breviculmis*, the same, and *C. gracilis*, otherwise found in Australia only.

The one genus marked SW? is *Ranunculus*, which is represented in Norfolk by *R. parviflorus*, an Australian species that does not reach New Zealand.

The three southern genera are (i) *Cardamine*, represented in the Kermadecs by *C. stylosa*, found from 60 to 820 miles in New Zealand, and therefore probably western; also found in Australia; (ii) *Veronica*, represented by *V. calycina*, an Australian species, in Norfolk, and by *V. salicifolia* in the Kermadecs, a very widely spread New Zealand type which probably reached the Kermadecs early; and (iii) *Luzula*, represented in Howe by the endemic *L. longiflora*, allied (?) to the New Zealand antarctic island *L. crinita*.

(12) The Kermadecs, Norfolk, and Howe contain a large number of endemic forms. It is clear that on the hypothesis of age and area, these should occur principally in the families and genera which have been in the



islands for the longest time, i. e. in those which are the most likely to have reached New Zealand. The endemics are:

TABLE VIII.

The letters K, N, H refer to the islands in which the families or species are found.

1. Magnoliaceae (NH)		19. Goodeniaceae (KH)	
1. <i>Drimys Howeana</i>	H	43. <i>Scaevola gracilis</i>	K
2. Cruciferae (KNH)		20. Epacridaceae (H)	
2. <i>Lepidium Howeiinsulae</i>	H	44. <i>Dracophyllum Filgéraldi</i>	H
3. Violaceae (KNH)		21. Myrsinaceae (KNH)	
3. <i>Hymenanthera latifolia</i>	N	45. <i>Rapanea kermadecensis</i>	K
4. Pittosporaceae (KNH)		46. <i>platystigma</i>	H
4. <i>Pittosporum bracteolatum</i>	N	47. <i>myrtillana</i>	H
5. <i>erioloma</i>	H	22. Sapotaceae (NH)	
5. Malvaceae (NH)		48. <i>Sideroxylon Howeanum</i>	H
6. <i>Abutilon Julianae</i>	N	23. Styracaceae (H)	
7. <i>Hibiscus insularis</i>	N	49. <i>Symplocos candelabrum</i>	H
6. Sterculiaceae (N)		24. Oleaceae (NH)	
8. <i>Ungeria floribunda</i>	N	50. <i>Nolelaea quadristaminea</i>	H
7. Rutaceae (KNH)		25. Apocynaceae (NH)	
9. <i>Melicope contermina</i>	H	51. <i>Alyxia Lindii</i>	H
10. <i>Evodia polybotrya</i>	H	52. <i>squamulosa</i>	H
11. <i>littoralis</i>	N	53. <i>gynopogon</i>	N
12. <i>Acronychia Endlicheri</i>	N	54. <i>Melodinus Baueri</i>	N
8. Meliaceae (NH)		26. Asclepiadaceae (NH)	
13. <i>Dysoxylum Patersonianum</i>	N	55. <i>Tylophora biglandulosa</i>	NH
14. <i>pachyphyllum</i>	H	27. Loganiaceae (H)	
9. Celastraceae (NH)		56. <i>Geniostoma petiolum</i>	H
15. <i>Elaeodendron curtispiculum</i>	N	28. Convolvulaceae (KNH)	
10. Sapindaceae (H)		57. <i>Ipomoea cataractae</i>	N
16. <i>Gutia coriacea</i>	H	58. <i>Calystegia affinis</i>	N
11. Leguminosae (KNH)		29. Solanaceae (KNH)	
17. <i>Carmichaelia exsul</i>	H	59. <i>Solanum Bauerianum</i>	N
18. <i>Millelittia australis</i>	N	30. Gesneriaceae (H)	
19. <i>Clianthus Baueri</i>	N	60. <b>NEGRIA RHABDOTHAM-</b>	
12. Saxifragaceae (H)		<b>NOIDES</b>	H
20. <b>COLMEIROA CARPODETOIDES H</b>		31. Myoporaceae (KNH)	
13. Myrtaceae (KNH)		61. <i>Myoporum obscurum</i>	N
21. <i>Aciclyptus Fullagari</i>	H	32. Plantaginaceae (H)	
22. <i>Metrosideros nervulosa</i>	H	62. <i>Plantago Headleyi</i>	H
14. Passifloraceae (NH)		33. Amarantaceae (NH)	
23. <i>Passiflora Baueriana</i>	N	63. <i>Achyranthes arborescens</i>	N
24. <i>glabra</i>	N	34. Piperaceae (KNH)	
15. Cucurbitaceae (KNH)		64. <i>Peperomia Baueriana</i>	N
25. <i>Bryonopsis affinis</i>	N	35. Chloranthaceae (K)	
26. <i>Melothria Baueriana</i>	N	65. <i>Ascarina lanceolata</i>	K
16. Araliaceae (KNH)		36. Lauraceae (H)	
27. <i>Nothopanax cissodendron</i>	H	66. <i>Cryptocarya Gregsoni</i>	H
28. <i>Meryta latifolia</i>	N	37. Thymelaeaceae (NH)	
29. <i>angustifolia</i>	N	67. <i>Pimelea congesta</i>	H
17. Rubiaceae (KNH)		68. <i>Wikstroemia australis</i>	N
30. <i>Randia stipulosa</i>	H	38. Santalaceae (NH)	
31. <i>Psychotria Carronis</i>	H	69. <i>Exocarpus homaloclada</i>	H
32. <i>Coprosma prisca</i>	H	70. <i>phyllanthoides</i>	N
33. <i>lanceolaris</i>	H	39. Euphorbiaceae (KNH)	
34. <i>putida</i>	H	71. <i>Euphorbia obliqua</i>	N
35. <i>pilosa</i>	N	72. <i>Norfolkiana</i>	N
36. <i>petiolata</i>	K	73. <i>Homalanthus polyandrus</i>	K
37. <i>acutifolia</i>	K	40. Urticaceae (KNH)	
18. Compositae (KNH)		74. <i>Celtis amblyphylla</i>	H
38. <i>Brachycome segmentosa</i>	H	75. <i>Ficus columnaris</i>	H
39. <i>Olearia Balli</i>	H	76. <i>Procris montana</i>	N
40. <i>Mooneyi</i>	H	77. <i>Boehmeria dealbata</i>	K
41. <i>Cassinia tenuifolia</i>	H	78. <i>australis</i>	N
42. <i>Senecio insularis</i>	H	79. <i>calophleba</i>	H

41. Orchidaceae (KNH)		92. <i>Clinostigma Mooreanum</i>	H
80. <i>Dendrobium gracilicaule</i>	H	93. <i>HOWEA BELMOREANA</i>	H
81. <i>Moorei</i>	H	94. <i>FORSTERIANA</i>	H
82. <i>brachypus</i>	N	95. <i>Rhopalostylis Baueri</i>	N
83. <i>macropus</i>	N	96. <i>Cheesemanii</i>	K
84. <i>Bulbophyllum argyropus</i>	N	47. Pandanaceae (NH)	
85. <i>Cleisostoma erectum</i>	H	97. <i>Pandanus Forsteri</i>	H
42. Amaryllidaceae (N)		98. <i>Freycinetia Baueriana</i>	N
86. <i>Crinum Norfolkianum</i>	N	48. Cyperaceae (KNH)	
43. Iridaceae (H)		99. <i>Mariscus haematodes</i>	NH
87. <i>Moraea Robinsoniana</i>	H	100. <i>Cladium insulare</i>	H
44. Liliaceae (KNH)		101. <i>Carex Neesiana</i>	N
88. <i>Rhipogonum dubium</i>	N	49. Gramineae (KNH)	
89. <i>Coralyline oblecta</i>	N	102. <i>Imperata Cheesemanii</i>	K
45. Juncaceae (H)		103. <i>Panicum Norfolkianum</i>	N
90. <i>Luzula longiflora</i>	H	104. <i>Poa polyphylla</i>	K
46. Palmaceae (KNH)		105. <i>Triticum Kingianum</i>	N
91. <i>HEDYSCEPE CANTER-BURYANA</i>	H		

These sum up to 105 species, belonging to 81 genera and 49 families. Of these 63 belong to 44 genera and 33 families which occur in New Zealand, while 38 species of 33 genera more belong to families that occur (though not the genera) in New Zealand. Thus, as predicted, the greater proportion occur in genera that have reached New Zealand, whilst nearly all the families that contain endemics have also reached that country, including the families (Saxifragaceae, Gesneriaceae, Palmaceae) that contain the endemic genera (*Colmeiroa*, *Negria*, *Hedyscepe*, *Howea*).

The families that contain endemics and do not reach New Zealand are Sterculiaceae, Celastraceae, Styracaceae (Symplocaceae), and Asclepiadaceae, each with one endemic species only, and represented, the first two each by the endemic species only in Norfolk Island, the third by the endemic only in Howe, and Asclepiadaceae by the endemic *Tylophora* in both Norfolk and Howe, and by *Vincetoxicum carnosum* and *Marsdenia rostrata*, both Australian species, in Howe.

The genera that contain endemics and do not reach New Zealand belong, all but these four, to families that reach New Zealand. They are *Abutilon*, *Ungeria*, *Evodia*, *Acronychia*, *Elaeodendron*, *Millettia*, *Guioa*, *COLMEIROA*, *Acicalyptus*, *Bryonopsis*, *Melothria*, *Randia*, *Psychotria*, *Scaevola*, *Symplocos*, *Notelaea*, *Alyxia*, *Melodinus*, *Tylophora*, *NEGRIA*, *Achyranthes*, *Cryptocarya*, *Wikstroemia*, *Baloghia*, *Homalanthus*, *Celtis*, *Ficus*, *Procris*, *Cleisostoma*, *Crinum*, *Moraea*, *HEDYSCEPE*, *Clinostigma*, *HOWEA*, *Pandanus*, *Panicum*, *Triticum*. Most are represented by one species only, but *Evodia* has two, *Alyxia* three, and *Boehmeria* three.

(13) It is evident, on my hypothesis, that the endemics should belong to the families that have been longest in the islands, i.e. on the whole to the largest families. In actual fact they belong to 49 families with 157 genera, or 3.2 genera per family, while the families that contain no endemics are 34 with 49 genera, or 1.4 genera per family. Twenty-three families with 3 or more genera contain 64 of them, 16 with 2 genera contain 16, and 44 with one genus contain 25 only.

(14) In the same way they should belong to the larger genera of the island floras. In actual fact, they belong to 81 genera which contain altogether 150 species, or 1.8 species per genus. The genera that contain no endemics are 125 with 152 species, or 1.2 species per genus.

(15) One will expect the island genera which contain endemics, inasmuch as they will on the whole be old, to be fairly large in New Zealand (when they reach there). There are 81 in all of them, and 37 do not reach New Zealand. The remaining 44 contain in New Zealand 362 species, or an average of 8.2 species per genus, while the average size of a genus in New Zealand is only 4.2 species. Even if the 362 be divided by the whole 81, the result is 4.4.

It is worthy of note, with reference to Professor Sinnott's hypothesis of 'swamping' (6, p. 214), that of the 362 species in New Zealand only 38 are wides. But on the other hand, the genera which are represented by wides in the islands are usually represented by wides in New Zealand.

(16) One will expect the families with endemics in the islands to be on the whole the same as the families with endemics in New Zealand, being the older families in each case. In New Zealand 76 families out of 91 contain endemics, but of these 18 (10, p. 359) are chiefly southern. In the islands 49 families contain endemics, and of these 38 are the same as in New Zealand. The other 11 include Sterculiaceae, Celastraceae, Styracaceae, and Asclepiadaceae, which do not occur in New Zealand, and also Cucurbitaceae, Goodeniaceae, Sapotaceae, Convolvulaceae, Amarantaceae, Piperaceae, and Amaryllidaceae, 7 families with only 17 species in all, and 15 of them wides, while the average range in New Zealand is only 587 miles (against a possible range of 1,080), all of them facts which point to the probably (comparatively) recent arrival of these families in New Zealand.

(17) In the same way, the genera with endemics in the islands should in general be genera that have endemics in New Zealand, or genera probably only comparatively recently arrived there. Of the 81 island genera with endemics, 37 do not occur in New Zealand, leaving 44 that do, of which no fewer than 38 possess endemics in New Zealand. It may be said, by those who have not worked at the matter in detail, that this is probably only the normal percentage of New Zealand genera that possess endemics, but in actual fact 95 genera out of 321 have no endemics in New Zealand, or a much higher percentage. The 6 genera that have no endemics in New Zealand are *Hibiscus*, *Ipomaea*, *Calystegia*, *Solanum*, *Peperomia*, and *Euphorbia*, which have in all 12 wides with an average range of 647 miles, and are thus small and slightly ranging genera, therefore probably on the whole young in New Zealand (in their affinity groups).

(18) One will expect the endemics of the islands to belong chiefly to the families that reach most islands.

TABLE IX.

Reaching	Kerm., Norf., Howe	22 fams.	50 gen.	69 spp.
	Norf., Howe	13	17	22
	Kerm., Howe	1	1	1
	Howe	10	10	10
	Norf.	2	2	2
	Kerm.	1	1	1

Thus much the best representation is among the families that reach all three groups of islands, the second best among those reaching two.

(19) Turning now to more general predictions, one will expect that the largest families in the islands will be those reaching three groups, then those reaching two, and lastly those reaching one only.

TABLE X.

Reaching	No. of fams.	1	2	3	4	Containing more genera
Three groups	27	3	8	5	2	5, 6, 6, 7, 8, 9, 9, 15, 18
Two groups	24	13	5	4	2	
One group	32	28	4	-	-	

Thus the families reaching three groups are 27 with 125 genera, or an average of 4.7, those reaching two are 24 with 43 (average 1.8), those reaching one are 32 with 36 (average 1.1).

(20) One will expect the same of the genera. In fact the 29 genera that reach three groups contain 73 species, or 2.5 per genus, while the 54 that reach only two groups contain 97 species (1.8 per genus) and the 123 that only reach one contain 132 species, thus completely fulfilling the prediction.

(21) Again, one will expect the families that reach three groups of islands, as oldest in their various affinities, to be best represented in New Zealand. The 27 families that reach all three groups of islands are represented in New Zealand by 79 out of 115 genera that reach New Zealand, the remaining 56 families only containing 36 genera.

(22) In the same way, the genera reaching all three groups of islands should be best represented in New Zealand. There are 30 of these, and 28 of them are represented in New Zealand, while of the remaining 176 only 87 are represented. The two genera reaching all three groups of islands and not reaching New Zealand are *Canavalia*, represented in all by *C. obtusifolia*, one of the stock plants of the drift of sea currents, and which one would expect in New Zealand, and *Boehmeria*, represented by a separate endemic in each of the three groups of islands.

(23) The species in common between three groups of islands, as oldest, should show the largest proportion of wides, and then those occurring in two or one groups.

TABLE XI.

<i>Occurring in</i>	<i>Wides.</i>	<i>Endemics.</i>
Three groups	23 (100 %)	—
Two groups	35 (90 %)	4
One group	139 (58 %)	101

Looking at the results that have been set forth in this paper, one is rather inclined to come to at least one important general conclusion. From the fact that by using age and area as a basis, 23 successful predictions have been made, many of which involve the mutual relationships of these islands and New Zealand in the matter of floras and their distribution, one may reasonably, I think, infer that age and area is as valid for these islands as for the mainland of New Zealand, not only as regards their local flora (considered entirely by itself) but as regards their floral relationships with New Zealand. But if the latter be the case, then it is clear that in all probability these islands must once have been a part of, or close to, the *land* communications over which the Indo-Malayan invasions of New Zealand travelled (cf. 5, p. 112). It is almost impossible to imagine them receiving by casual transport across the water a flora which would show such striking numerical relationships, and relationships in such detail, to the flora of New Zealand. One cannot imagine that age and area should hold for the *mutual* relationships of two areas that were peopled with plants by independent invasion of casual waifs across the ocean. Taking these facts together with those set forth for New Zealand on p. 473, above, one may, I think, come to the conclusion that the peopling of New Zealand from Indo-Malaya was by land (not, of course, necessarily absolutely continuous, but with at most comparatively narrow straits), and that the islands which have been dealt with in this paper formed part of, or were very near to, that land.

I am much indebted to the Director of Kew Gardens, Sir David Prain, C.M.G., F.R.S., for references to literature, and to my daughter Margaret for Diagram 3.

## SUMMARY.

In this paper age and area is applied to the floras of the islands outlying between New Zealand and the nearest larger areas of land to the north or north-west. It proves to be equally applicable to them, both for themselves and for their relationships to New Zealand, showing that they must in all probability have formed part of a land mass or masses running down to New Zealand from Indo-Malaya. Lists are given of the New Zealand genera (Table II), showing the invasions of which each may be supposed to have formed a part, deduced from their local distribution in New Zealand; of the genera of the islands (Table III), showing those that reach New Zealand; and of the endemics of the islands (Table VIII). It is shown that the

invasions of New Zealand were probably four—northern, Kermadec, western, and southern—the western probably arriving by the ridge on which stand Norfolk and Lord Howe.

Applying the usual method of prediction and verification, it is then shown that (1) a large part of the floras of these islands also occurs in New Zealand; (2), (3), and (4), genera and families not represented in New Zealand by actual species, are represented by endemics or by Australian and Polynesian wides in the islands; (5) more than half the species not found in New Zealand are endemic to the islands, and (6) the rest are Australian and Polynesian wides; (7) these species belong very largely to genera and families that do reach New Zealand; (8) families and genera that have reached New Zealand are better represented on the islands than those that have not; (9) the families that do not reach New Zealand at all are all small and little distributed in the islands; (10) the genera that do not reach New Zealand belong mainly to families that do; (11) the bulk of the island floras belongs to genera and families of the northern, Kermadec, and western invasions of New Zealand; (12) the island endemics occur chiefly in genera and families that have reached New Zealand; they occur chiefly in (13) the larger families of the islands, and (14) the larger genera; (15) the island genera with endemics are usually large in New Zealand; (16 and 17) the island families and genera with endemics are in general the same as in New Zealand; (18) the island endemics belong chiefly to the families that reach three islands; (19) the largest families in the islands are those reaching all three, and (20) similarly the largest genera; (21) the families that reach three are best represented in New Zealand, and (22) similarly the genera; (23) the species in common between three islands show the largest proportion of wides, then those from two and from one.

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# Contributions towards a Knowledge of the Anatomy of the Genus *Selaginella*.

## The Root.

BY

J. C. TH. UPHOF, PH.D.

With thirteen Figures in the Text.

THE author wishes to make an attempt to give an account of the anatomy of the root of the genus of *Selaginella*, under which he includes also the so-called rhizophores. In various excellent articles in 'Annals of Botany', the anatomy of the stem, leaf, and ligule of this genus have been studied by Gibson, and the strobilus by Miss Mitchell. This leaves the root of the genus untouched. The writer has made his observations at the Royal Botanical Gardens at Kew, where most of his studies have been done at the Jodrell Laboratory, and is therefore thankful for the kind help he received from Sir David Prain, Director of the Botanical Gardens, and from Mr. L. A. Boodle, Curator of the Jodrell Laboratory; also to Mr. W. Emery for preparing the manuscript for publication.

The present species of the genus of *Selaginella* need some consideration; the author considers them as remnants of older periods (they existed already in the Palaeozoic Flora) in which related genera reached their highest pitch of development. The origin and morphological development of their organs will be still better understood when fossil forms have been properly studied, which no doubt will be the case in the near future. It has to be considered that Selaginellas and the present living related families are the most primitive forms of vascular plants, and therefore morphologically of the utmost importance. Especially in the fossil species and monstrosities of plants of present forms there may be some likelihood of studying the morphological origin of one of the most important organs of the higher plants, namely, that of the root.

The present living distant relatives of the Selaginellas are the Lycopodiaceae and Psilotaceae; the former are in possession of true roots, whose anatomical construction is much like that of the stem; on the other hand, the latter family has no true roots, but as a substitute has subterranean shoots. The genus *Isoetes*, which is at present the only representative of



the family of the Isoetaceae, also shows in some of its characteristics slight resemblance to *Selaginella*, and possesses true roots. Under the fossil forms, *Stigmaria* has been extensively studied; especially Scott (11) has greatly distinguished himself; he considers the subterranean organs of this genus as a primitive stage in the differentiation of root and shoot.

The aerial roots of *Selaginellas* have been considered by various investigators as leafless shoots, as roots, and as organs standing between root and shoot; others consider them as special organs (1).

The first investigators who have drawn considerable attention to the aerial roots are Leitgeb (8) and Nägeli, who call them *Wurzelträger* (= rhizophores) and consider them morphologically as stem-like, leafless organs, which only perform the function of bearing true roots; both investigators consider it as of much importance that the rhizophores have no root-cap and therefore could not be roots; on the other hand, there are nevertheless roots, whose morphological significance is beyond doubt, which do not develop any such root-caps, as has been studied under the Hippocastanaceae and Sapindaceae by Waage (15). Also Pfeffer (9), Treub (14), Bruchmann (2), Fries (5), Campbell (3), and Worsdell (17) consider them as leafless stems. On the other hand, van Tieghem (12), Douliot (13), and Sarauw (10) suggest they are roots; whereas Goebel (6 and 7) supposes they have the characteristics of both; also Bower (1) states that the structure is like that of a root, but that in some cases the rhizophore has a structure resembling that of an axis. Those who are in favour of a stem-like nature, such as Pfeffer (9), Bruchmann (2), and Worsdell (17), claim that sometimes leafy shoots are seen instead of leafless rhizophores, although on the other hand it has been observed that true roots may give rise to branches, as in *Anthurium longifolium*, *Asplenium esculentum*, and *Neottia Nidus-avis* (16).

Generally the root of the genus *Selaginella* is simple in construction and differs from the stem. Although there is much discussion as to the morphological value of the rhizophore, as has been shortly outlined above, the writer does not hesitate to consider them as roots which have been developed in the air instead of in the soil, and have therefore had to adapt themselves to their particular environment, which necessitated a change in some of the tissues. Instead of being called rhizophores, the name 'air-roots' would therefore be more appropriate; this name will be frequently used in this sense in the present article.

Both kinds of roots have been examined by the author in 262 different species, of which about forty have been studied anatomically.

There is very little prospect of arranging this genus phylogenetically in accordance with the appearance of the roots. The aerial roots are always exogenous in origin, whereas the branching of the roots in the soil is monopodial, although apparently dichotomous. There is apparently more diver-

sity in the location of the roots of the air than those developed in the soil. The former have their origin always at the forking off of an ordinary branch, either at the upper or lower side of the stem; they may be found over the entire length of the stem, e.g. *S. Kraussiana*, *S. Bakeriana*, and *S. serpens*; in other species they are only seen below the middle of the stem, as in *S. atrovirens* and *S. viticulosa*. Xerophytic species such as *S. cuspidata*,

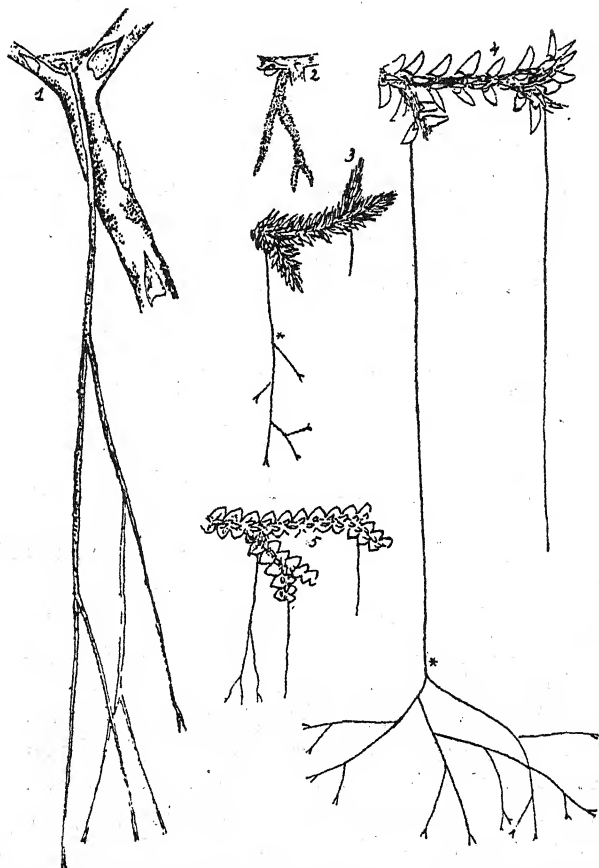


FIG. I. Different types of rhizophores or aerial roots. 1, *S. Wildenowii*; 2, *S. pilifera*; 3, *S. rupestris*; 4, *S. rubella*; 5, *S. serpens*.

*S. pilifera*, and *S. lepidophylla*, only form aerial roots towards the base of the stem when they are grown in a very moist environment, which also may be said of the hygrophytes, *S. Vogelii*, *S. caulescens*, and *S. viridangula*. On the other hand, the xerophytic species, *S. sanguinolenta*, forms under natural conditions aerial roots at several places of the entire stem.

The thickness of the aerial roots, which differs but slightly in the same species, is fibre-like (about  $\frac{1}{4}$  mm. in thickness) in *S. molliceps*, *S. Bakeriana*, *S. apus*, and *S. haematodes*; they are string-like (about 1 mm. and more in

thickness) in *S. rubella*, *S. Martensi*, and *S. decora*; while those of *S. Wildenowii* are as thick as 2.5 mm. and sometimes slightly more in diameter.

The air-root of some species practically never branches before entering the soil, e.g. *S. uncinata*, *S. rubella*, *S. formosa*, and other species; on the other hand, those of other species may branch when in the air, e.g. *S. Wildenowii*, *S. rupestris*, *S. rupincola*, and *S. inaequalifolia*.

The length of the rootlets and entire root system in the soil depends greatly upon the presence of plant food, and also stands in close relation to the size of the air-roots, whose length may be considerable in some species, as is summarized in the following list:

*Length and diameter of air-roots of various Selaginellas.*

Name of Plant.	Length.	Diam.	Name of Plant.	Length.	Diam.
	mm.	mm.		mm.	mm.
<i>S. molliceps</i>	6-15	$\frac{1}{4}$ - $\frac{1}{2}$	<i>S. Bakeriana</i>	6-12	$\frac{1}{4}$ - $\frac{1}{2}$
<i>S. patula</i>	10-18	$\frac{1}{4}$ - $\frac{1}{2}$	<i>S. uncinata</i>	35-40	$\frac{1}{4}$ - $\frac{1}{2}$
<i>S. decora</i>	30-45	$\frac{1}{4}$ - $\frac{1}{2}$	<i>S. inaequalifolia</i>	20-40	$\frac{1}{4}$ - $\frac{1}{2}$
<i>S. viticulosa</i>	3-8	$\frac{1}{4}$ - $\frac{1}{2}$	<i>S. Kraussiana</i>	25-35	$\frac{1}{4}$ - $\frac{1}{2}$
<i>S. Wildenowii</i>	90-260	1-2 $\frac{1}{2}$	<i>S. apus</i>	5-12	$\frac{1}{4}$ - $\frac{1}{2}$
<i>S. cuspidata</i>	5-8	$\frac{1}{4}$ - $\frac{1}{2}$	<i>S. Douglasi</i>	20-28	$\frac{1}{4}$ - $\frac{1}{2}$
<i>S. pilifera</i>	5-8	$\frac{1}{4}$ - $\frac{1}{2}$	<i>S. grandis</i>	8-12 (20)	$\frac{1}{4}$ - $\frac{1}{2}$
<i>S. rupestris</i>	4-8	$\frac{1}{4}$ - $\frac{1}{2}$	<i>S. Galeotti</i>	80-120	$\frac{1}{4}$ - $\frac{1}{2}$
<i>S. serpens</i>	6-12	$\frac{1}{4}$ - $\frac{1}{2}$	<i>S. formosa</i>	40-60	$\frac{1}{4}$ - $\frac{1}{2}$
<i>S. Martensi</i>	30-60	$\frac{1}{4}$ - $\frac{1}{2}$	<i>S. haematodes</i>	6-15	$\frac{1}{4}$ - $\frac{1}{2}$
<i>S. rubella</i>	30-65	$\frac{1}{4}$ - $\frac{1}{2}$	<i>S. sanguinolenta</i>	4-8	$\frac{1}{4}$ - $\frac{1}{2}$

Anatomically there is a marked difference between the air-roots and terrestrial roots on one hand, and the stem (especially of the heterophyllous species) on the other hand. Of the species whose anatomical construction is described below many sections of roots in various stages of development have been made, but no lacunae and no trabeculae, which are characteristic in stems of all heterophyllous species, have ever been encountered, and these sections show a very important differentiation as far as the anatomy is concerned. For this purpose longitudinal sections of air-roots with attachment of the stem were made by the writer from several species. Fig. IV, 1, shows such a section of *S. rubella*, in which the difference between the two vascular bundles of both kinds of plant organs is clearly visible; the bundle of the stem is above, that belonging to the air-root being on the left of the drawing. There are, however, according to some investigators, indications of the transformation of such aerial roots into leafy shoots, which would apparently prove that these organs are no real roots but shoots. Bruchmann (2) has observed in *S. Kraussiana* that when individuals which have been outside during the summer are in autumn put into a warmer environment these roots form at the apex stems with small leaves, as has been stated above; also that other plants of other families may form leafy branches at their roots. Also Pfeffer (9), and later Worsdell (17), have

made observations that, instead of so-called rhizophores, which had been expected, they found small-leaved shoots in their place, thus apparently proving that rhizophores are of stem-like origin. No author, however, seems to have made a comparative anatomical and physiological study of such leafy shoots and rhizophores or aerial roots.

In *S. uncinata* I have frequently observed true (although thin and small) twigs instead of roots or so-called rhizophores. These twigs bear small leaves in proportion to their size. The leaves are always at some distance from each other, such shoots having thus the appearance of the main stems. In one instance I noticed that such an organ was deprived of its leaves on a length of about 12 mm., which is rather considerable for such a thin shoot, the remaining part being further covered with leaves as described above. Such shoots branch in the usual way, producing at the proper places aerial roots. Without any further anatomical study one would be apt to suppose that the leafless part would have the construction of a rhizophore and the leafy part that of a stem. Such modifications, however, we only observe where normal twigs have been damaged or bruised in some way or other. Often it could be observed in *S. uncinata* (Fig. II, 1, A), and the writer noted it a few times in *S. grandis* (Fig. III, 5), *S. inaequalifolia*, *S. Wilde-nowi*, *S. Kraussiana*, *S. serpens*, and once in *S. rubella*, *S. Bakeriana*, and *S. Douglasi*.

The anatomical construction of such shoots is exactly the same as that of the ordinary stem. The lacunae and trabeculae, which are never formed in the aerial roots, are present in the shoots as mentioned above, although smaller, in proportion to their size, than those in the larger stems. That part of the shoot which is leafless for a distance of 12 mm. also contains lacunae, and consequently some trabeculae, and is a long internode. Moreover, I observed in all cases that such a long internode is already positively heliotropic in its early youth, whereas the aerial root is negatively heliotropic from its start. For a further comparison, the following drawings in Fig. II relating to *S. uncinata* are useful. 1 is an old shoot which has been bruised, and soon formed two little twigs, A, A<sup>1</sup>, which according to some investigators are rhizophores transformed into leafy branches; A<sup>1</sup> has formed in the usual way one aerial root, B; 2 is a section of the so-called metamorphosed rhizophore which has, as stated above, the same construction as an ordinary stem, although the trabeculae are somewhat shorter; also the further part of the vascular bundle is the same as that of a stem; 3 is a part of the main stem, drawn for comparison; 4 is a cross-section of aerial root B; 5 is the epidermis and hypodermis of an ordinary aerial root, and 6 that of rhizophore B; 7 is a cross-section of an ordinary young aerial root, 8 of a root developed in the soil, 9 and 10 the epidermis of a stem and of an aerial root; and 11 shows the branching system of a root, entering the soil at \*.

An intermediate, as far as anatomical construction is concerned, between rhizophore and stem has not been observed, although the writer studied thirty-five such so-called transformed rhizophores of different species. Moreover, they are all distinctly positively heliotropic from the very start, while on the contrary all rhizophores or aerial roots, as has been stated, are

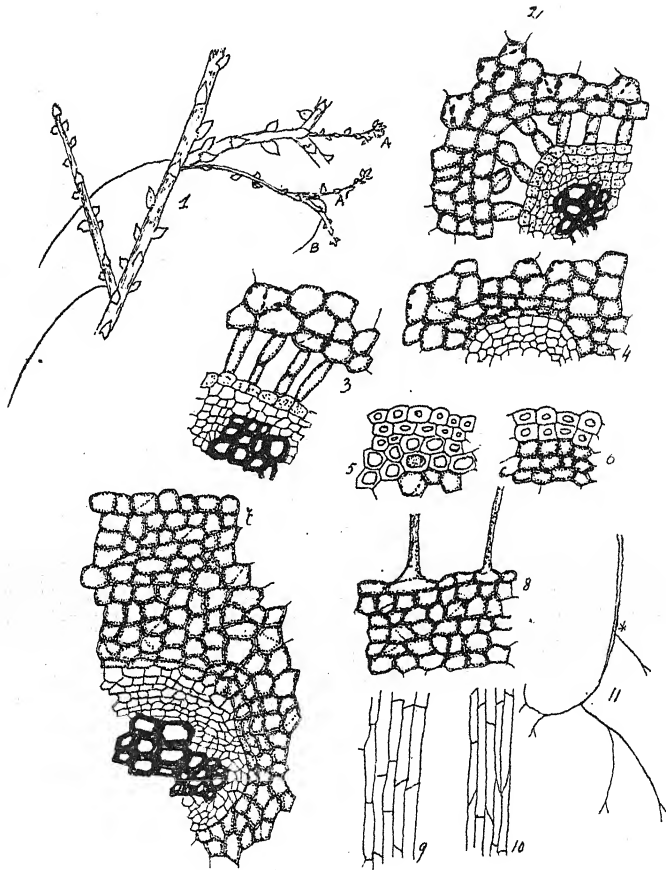


FIG. II. 1, stem of *S. uncinata*: A, branches instead of rhizophores; B, rhizophore formed by such a branch; 2, cross-section of stem A in 1; 3, cross-section of ordinary stem; 4, cross-section of rhizophore B in 1; 5, epidermis and hypodermis of ordinary rhizophore; 6, the same of rhizophore B in 1; 7, cross-section of young rhizophore; 8, cross-section of a young root with root-hairs; 9, epidermis of stem; 10, the same of rhizophore; 11, branching system.

negatively heliotropic, an argument in favour of their root nature which is beyond doubt.

The same indications are shown in Fig. III, 3–8, in *Selaginella grandis*, which are even more characteristic than in *S. uncinata*; as the stem of the former has a strongly developed system of vascular bundles which manifests itself also in the small shoot, whereas the rhizophore and root have a small

vascular bundle of which the xylem consists of only eight to nine tracheides having only one protoxylem.

The negative heliotropism of both types of roots has been studied in

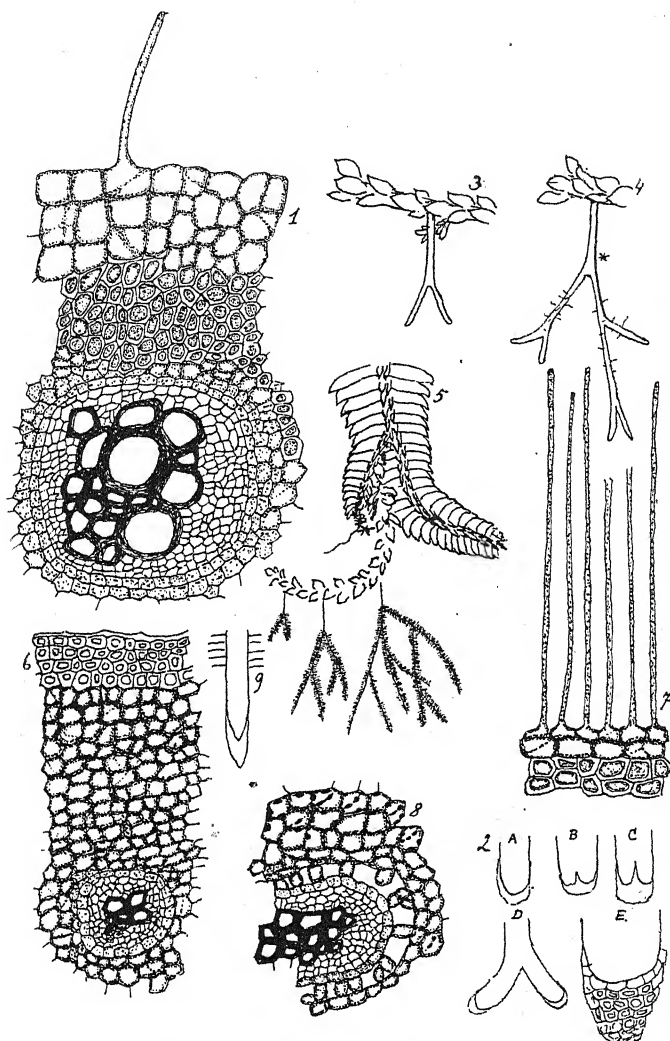


FIG. III. 1, cross-section of root of *S. Wildenowii*; 2 (A-E), development of root of same species; 3-9, *S. grandis*; 3, aerial root without root-hairs or root-cap; 4, aerial root from \* downward with root-hairs and root-cap; 5, branch developed in extreme moisture, \* branch instead of rhizophore, which has formed an aerial root; 6, cross-section of rhizophore; 7, part of rhizophore with root-hairs; 8, cross-section of branch which is in the place of rhizophore; 9, root with root-cap and root-hairs.

*S. Kraussiana*, *S. Wildenowii*, *S. rubella*, *S. molliceps*, *S. pallida*, *S. serpens*, and *S. inaequalifolia*. Young shoots of these species were turned upside-down; the very young aerial roots soon turned in the opposite direction to

that of the incident light ; when the darkest part was on the left of the stem, the aerial roots turned themselves in this direction ; on the other hand, the true shoots always tend to grow positively heliotropic. Of old or partly full-grown rhizophores, the growing-point always develops towards the darkest side again after having been moved.

Diversified directions of various aerial roots can be readily observed on strongly branching Selaginellas, which grow intermingled with other densely growing plants and thus do not get an equal distribution of light. On such plants one finds these roots growing in all directions from vertical to horizontal, according to the effectiveness of the entering light.

Plants which were grown in hanging baskets in a light part of a greenhouse show in their rhizophores various degrees of sensitiveness to light. Rhizophores or aerial roots which are hanging from the stem between the glass of the greenhouse and the basket in which the plant grows are strongly curved towards the basket ; on the other side, where the light is less intense on account of the presence of other plants, the curvature of these aerial roots is less marked ; thus the degree of the sensitiveness is not so pronounced, and consequently the rhizophores are less curved in the direction opposite to the light. The above facts were observed by the author with *S. uncinata*, *S. Lobbi*, *S. Watsoniana*, and *S. Martensi*. In a practically similar way the negative heliotropism was demonstrated by putting parts of stems with young rhizophores of *S. grandis*, *S. serpens*, *S. haematodes*, and *S. Bakeriana* in watch-glasses which were covered with a glass plate with black paper above it except for a narrow margin towards the window side of the laboratory in which they were placed. The top of the rhizophore or aerial root was placed in such a way that it was touched by the light. After eight days the apex of these rhizophores had all partly developed in the opposite direction to that of the incident light, whereas the young shoots were growing towards the light.

When aerial roots enter the ground, their one-sided growth is not as strongly marked as if they were growing in the air, as the opposition of the light is overcome as soon as they enter the soil. Their growth is more or less spreading in all directions, depending only upon the moisture and the presence of plant food in the substrata. In the soil they branch monopodially in the same way as they would do in the air.

Aerial roots which are exposed to the light contain in their cells a considerable amount of chloroplasts, which are absent in the roots growing in the soil ; such organs of *S. Wildenowi*, *S. rubella*, and *S. Lobbi*, which were afterwards exposed to the light, became greenish and developed chloroplasts, especially in the cortex.

Some anatomical difference exists between the aerial root and the terrestrial root ; the former, on account of its environment, is surrounded by a thick-walled epidermis and hypodermis, and sometimes one or two layers of



the outer cortex are thick-walled, which is caused by secondary layers in the cell-walls. Treatment with potassium hydroxide gives a yellow colour and suggests the presence of suberin; its amount depends upon the exposure; organs developed in a moist and shady climate contain considerably less suberin than those grown in a dry exposed place.

Rhizophores or aerial roots which approach the soil become even more thin-walled, and having touched the soil the cell-walls are only composed of their primary walls. Such roots, soon after entering the soil, form root-hairs. Such behaviour was studied by the writer on *S. Lobbi*, *S. rubella*, *S. Wilde-nowi*, *S. Kraussiana*, *S. Douglasi*, and *S. Martensi*. Of the latter species, the following observations may be made: The aerial root has a small-

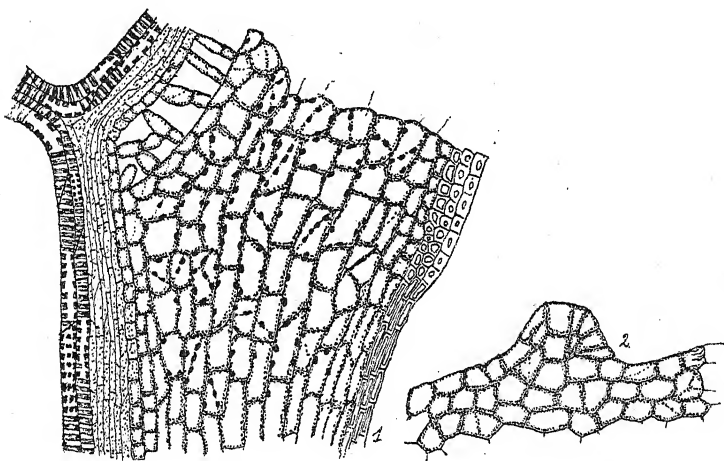


FIG. IV. *S. rubella*. 1, junction of vascular bundles of rhizophore and stem; 2, young rhizophore.

celled and rather thick-walled epidermis; the cells of the hypodermis are also thick-walled; the outer rows of the cortex show some secondary thickening of their walls, and in very exposed places these cell-walls of the cortex may be considerably thicker (Fig. V, 3). When the aerial root approaches the moist soil, at a distance of about 2 millimetres, its structure soon shows some differences, e.g. the epidermis is not as thick-walled, and the space in the cells is larger; further, there is no great difference in size between the cells of the epidermis and of the hypodermis (Fig. V, 4), such difference as exists having no doubt been caused by the moisture of the soil; there is, however, no difference in the cells of the cortex, whether developed in the air or near the surface of the soil. When the aerial root actually touches the soil the epidermis does not show any secondary layers in its walls, although they are still present in those of the hypodermis; the epidermis, however, is often followed by another, sometimes two layers of

cells which are thin-walled, and which the author considers to have originated from the epidermic cells, as when very young these cells lie in radial direction towards one another. Sometimes it happens that the epidermis of such a root has formed a few root-hairs; but after having penetrated the soil the root-hairs are abundantly formed, not, however, from every epidermic cell, e.g. in the case of *S. lepidophylla* or *S. pilifera*.

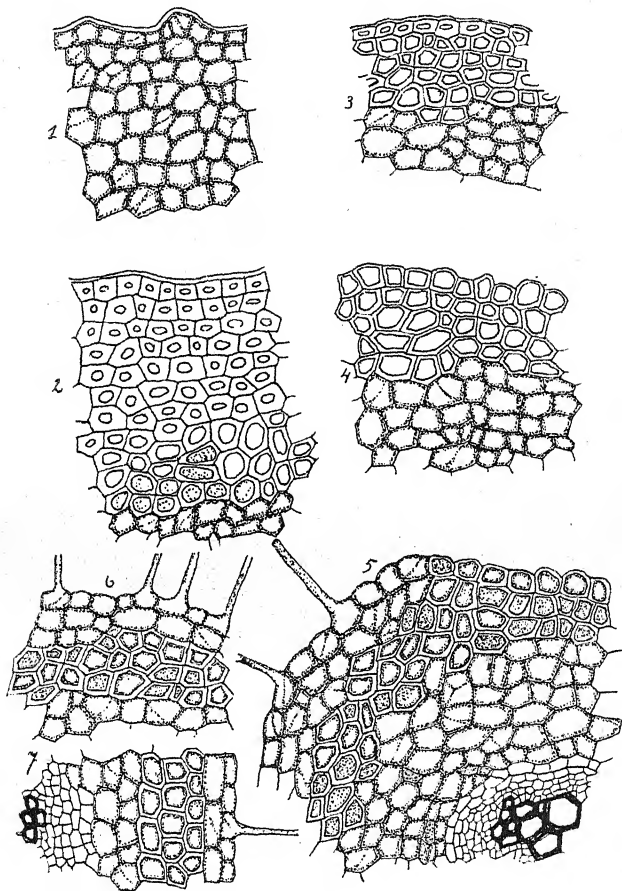


FIG. V. 1, 2, *S. Bakeriana*: 1, part of young rhizophore; 2, old rhizophore. 3-7, *S. Martensi*; 3, rhizophore surrounded by air; 4, rhizophore approaching the soil; 5, rhizophore touching the soil, showing habit of aerial root and terrestrial root; 6, 7, root developed in the soil.

Anatomically and physiologically interesting was the following experiment. Growing rhizophores, which were 3 to 10 millimetres distant from the soil, were covered lengthwise for one half with wet soil, the other side being exposed to the air; after ten days the latter part had a construction like a root developed in the air, while that part which was grown against the soil had the same appearance as a terrestrial root, e.g. thin epidermis

walls with root-hairs followed by another thin-walled layer and then by a three-layered hypodermis composed of somewhat thick walls, before one observes the cortex proper, this being the regular succession of tissues found in practically all roots with which the writer has worked. Near the boundary where the rhizophore partly touches the wet soil and partly the free atmosphere, one is able to find any modification between the aerial and terrestrial root, due to the direct action of environment. Both types can be observed in Fig. V, 5-7.

The writer suggested above that the thin-walled layers of a terrestrial root which underlie the epidermis have been formed from the latter, whereas the somewhat thick-walled layers belong to the hypodermis. Such can clearly be observed on *S. viticulosa*, the aerial roots of which have a red-coloured epidermis and hypodermis, the colour pigment being encrusted in the secondary walls. The same colour can be observed in the thick-walled layers of the terrestrial root, although its colour is not present in the thin walls of the epidermis cells, on account of the absence of secondary layers. Moreover, a longitudinal section of an aerial with a terrestrial root shows the connexion of the hypodermis in both. The author is unable to understand why these cells of hypodermis are thick-walled in a terrestrial root.

In twenty-two instances roots of *S. rubella* were grown in the soil and, still being connected with the plant, were carefully taken out of the soil, washed off, and kept so that they were unable to touch the soil; some of these rapidly died; in two instances the roots continued their growth in the air, their root-caps gradually consisting of less cells and finally disappearing; at last this part of the root became in construction again entirely an aerial root.

The above anatomical and physiological investigations are not in favour of the stem habit of the rhizophores, which are on the contrary entirely root-like in construction and behaviour. In one of the above-mentioned experiments it could not be stated that the half of the rhizophore which was open to the air was a stem, while the half which was surrounded by soil was a root.

All roots possess a monarch vascular bundle, the endodermis of which is in some species difficult to distinguish; it is very clear in *S. Wildenowii* and *S. rubella*, but does not show very well in *S. serpens*, *S. Bakeriana*, and some other species. No root, whether grown in the air or in the soil, ever develops any lacunae or trabeculae. The pericycle is composed of one layer. The elements of the phloem, although not as abundant as in the vascular bundle of a stem, are arranged in the same manner as in the stem, and can to a certain extent be compared with the studies of Gibson on the anatomy of the stem. The sieve-tubes are consequently present, although apparently not much developed near the protoxylem. The phloem surrounds the xylem entirely. This xylem possesses but one group of proto-

xylem (the vascular bundles of a stem have more), and is strongest developed in the oldest part of the aerial root of *S. Wildenowii*. The tracheides of the metaxylem differ greatly in number in the various species; they are abundant in *S. Wildenowii* and *S. rubella*, while only a few are to be found in *S. serpens*. The types of tracheides are the same as in the stem.

The author observed that a root-cap is present on all roots which are in possession of root-hairs, whether these roots have been developed in the air or in the soil. On the other hand, roots which do not have such root-hairs, as is the case with the so-called rhizophores, are correlated with the absence of a root-cap. As the writer demonstrated, some species may lose the root-cap when terrestrial roots are forced to develop in the air; on the other hand, the author was unable to force plants which develop roots with root-hairs and root-cap in the air, such as *S. grandis*, to form in some way or other roots without root-hairs or root-cap; although *S. grandis* may form in a dry environment aerial roots without root-hairs.

The root-cap and root-tip are very simple in construction, and the development has already been studied by Treub (14), van Tieghem (13), and other investigators. The root-cap is pointed in *S. grandis*; in *S. Bakeriana*, *S. rubella*, and *S. Wildenowii* it is rounded, and is especially well developed in the latter species. In homophyllous species, e.g. *S. rupestris*, *S. rupicola*, and *S. densa*, it is least developed and is composed of but a few cells.

Before giving a description of the anatomy of roots of various species of *Selaginella* the author wishes to state that he has made no study of this organ in the young sporophyte. An account of the development and comparative study of the latter will be given in another publication.

#### *Selaginella Wildenowii*.

The aerial roots or rhizophores are in this species strongest developed of all (Fig. I, 1). They attain a length of 90 to 260 millimetres, the diameter ranging from 1 to about 2.5 millimetres. They are red in colour, the pigment being dissolved in the vacuoles of the cells, especially those of the hypodermis and cortex. They may be developed at any distance up the plant.

The epidermis is composed of smaller cells than the hypodermis and soon becomes thickened by secondary layers. The hypodermis is composed of three to four layers of cells. The following cells of the cortex are considerably larger than those of the former tissues, and are thin-walled and usually composed of twenty to twenty-five rows of cells in the old rhizophores or about fifteen to twenty rows in those of the first branching (Fig. VI, 1-5, and Fig. VII). The endodermis is well developed in the main rhizophore, the cell-walls in young specimens showing a varying degree of

thickness in which suberin can be demonstrated. The protoxylem is highly developed in the oldest part of the rhizophore, before it branches off for the first time; it is composed of from twenty-five to even forty tracheides; in young organs the secondary thickening of the walls takes place at the outer side of this group of tracheides, as is shown in Fig. VII;

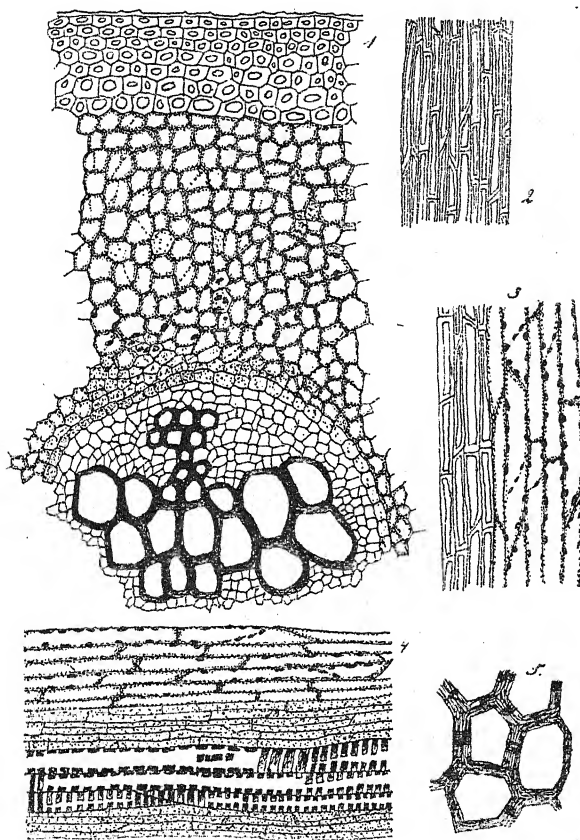


Fig. VI. *S. Wildenowii*. 1, cross-section of rhizophore; 2, longitudinal section of epidermis and hypodermis; 3, longitudinal section of thick- and thin-walled cortex; 4, longitudinal section of cortex, endodermis, pericycle, protophloem, phloem, parenchyma, and metaxylem; 5, cross-section of tracheides of xylem.

when the rhizophore has branched off the protoxylem usually consists of six to ten tracheides. The metaxylem is always strongly developed, especially in the old primary rhizophore; its tracheides are broad. The entire vascular bundle of the primary rhizophore is kidney-shaped (Fig. VII); when this organ has branched off the bundle is roundish (Fig. VI, 1).

The terrestrial root possesses an epidermis with large cells which occasionally lengthen towards the periphery into root-hairs; the epidermis

is followed by two layers of thin-walled cells which are of the same appearance. The next tissue met with is the hypodermis, which is composed of about three to five layers of cells; the walls are thick on account of the presence of secondary walls. The cortex is thin-walled, though sometimes its outer cell-layer may be slightly thick-walled. The endodermis is not as

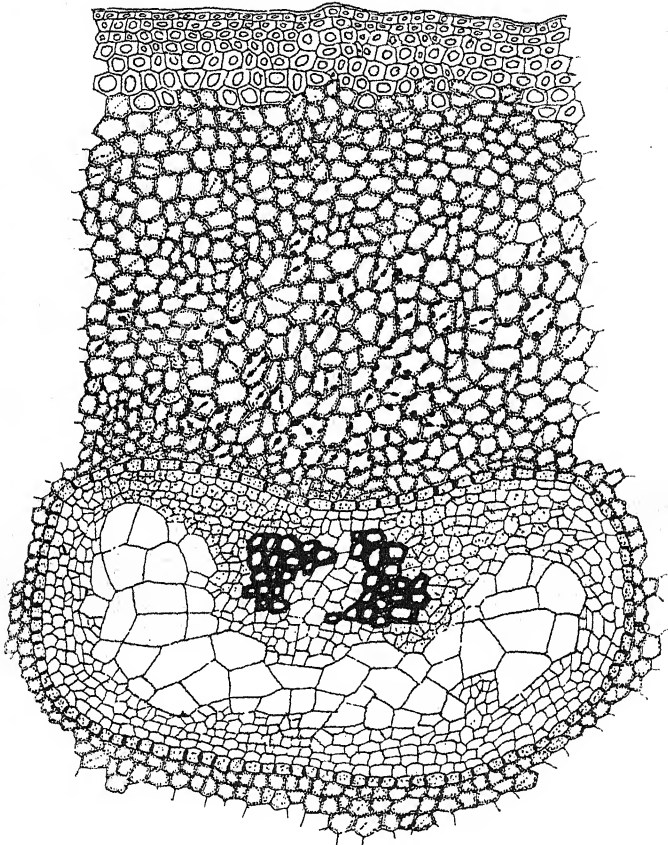


FIG. VII. Section of the top part of a young rhizophore of *S. Willdenowii*.

easily visible as that of the primary air-root or rhizophore. The phloem contains but few sieve-tubes in comparison with the aerial root. The xylem is the same as has been described above.

#### *Selaginella rubella*.

The aerial root reaches a length of 30 to 65 millimetres and a diameter of  $\frac{3}{4}$  to 1 millimetre. The epidermis has rather a thick-walled cuticle, and the other walls of the epidermis and hypodermis are thick, those of the former differing but slightly in size from those of the latter, except that

hypodermis cells belonging to the inner row are sometimes larger. The outer layer of the cortex also may be slightly thick-walled, although the remainder are thin-walled, and are composed of eight to ten rows of cells. The cells of the endodermis are much smaller than those of the inner cortex, and therefore easily visible. The phloem is much like that of the stem, and therefore does not need any further description. The xylem is composed of

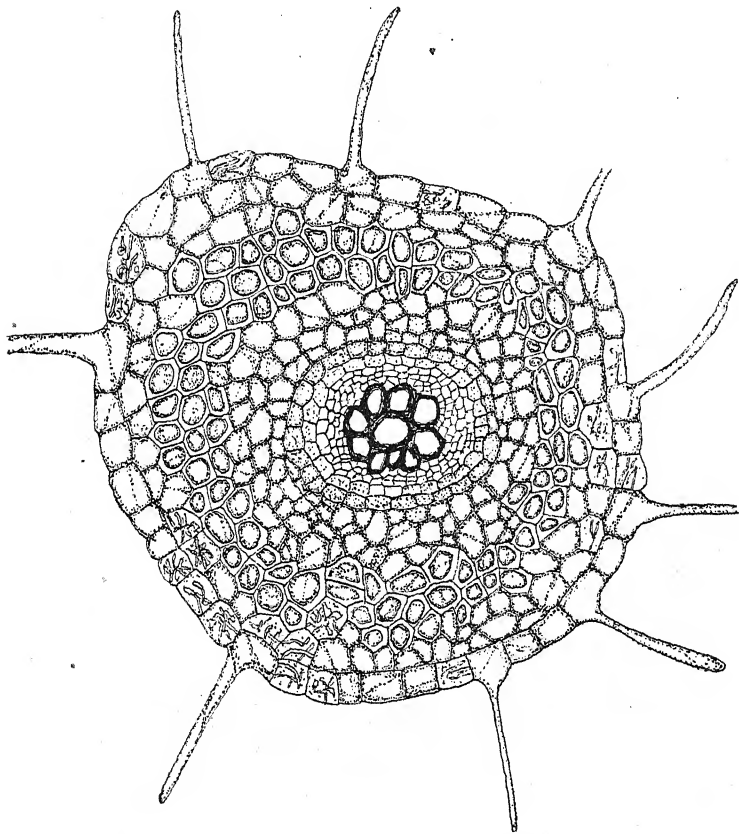


FIG. VIII. Cross-section of root of *S. rubella*.

thirty-two to forty tracheides; the members of the protoxylem, although visible, are not much smaller than those of the metaxylem.

All cells contain a red pigment which is dissolved in the vacuole. The cells of the hypodermis, cortex, and sometimes of the epidermis contain mycelia, which suggests the presence of mycorrhiza (Fig. IX, 1 and 2). No further study of this mycorrhiza has been made, as it would require a special treatise.

The terrestrial root (Fig. VIII) has a large-celled epidermis, and cells of the two following layers also have the same appearance. The hypodermis



is composed of three rows of cells, which are slightly thick-walled through secondary layers; the thin-walled cortex is composed of three to four layers. Of the vascular bundle the phloem is in comparison stronger developed than the xylem; the latter is composed of eight to ten tracheides, of which

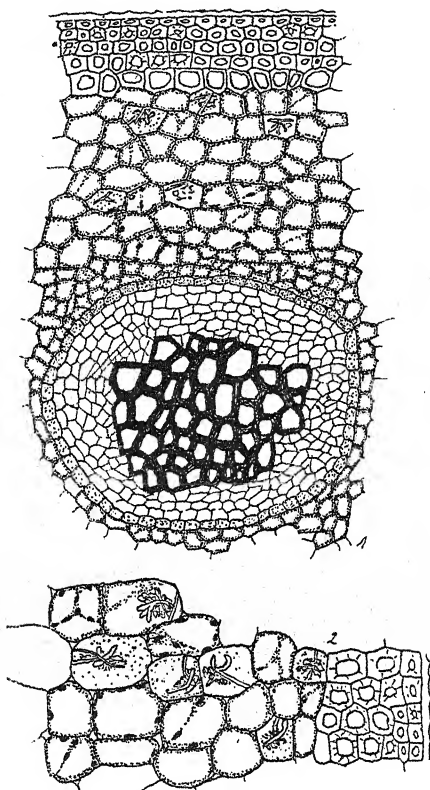


FIG. 1X. *S. rubella*. 1, cross-section of rhizophore; 2, longitudinal section: some cells show mycorrhiza.

about three to four belong to the protoxylem. Also in the root mycelia have been found.

#### *Selaginella haematodes*.

The rhizophores are fibre-like, 6 to 10 millimetres in length and  $\frac{1}{4}$  to  $\frac{1}{2}$  millimetre in diameter. They only develop towards the base of the stem and never higher up. The epidermis, hypodermis, and cortex cells do not differ much from one another in size. The epidermis has a very thin cuticle, and with those of the hypodermis shows but slightly secondary walls, probably because they are formed very close to the moist soil. The cortex is composed of four to seven rows of cells. The vascular bundle is small,

and its xylem is but little developed, being composed of seven tracheides, of which four belong to the protoxylem.

The roots are very thin; only a few cells of the epidermis are elongated into hair-roots. Then follow two layers of thin-walled cells, then the hypodermis cells composed of three layers which are but very slightly thick-walled. The vascular bundle is very small—the writer counted from two to three sieve-tubes in the phloem and but four to five tracheides in the xylem.

*Selaginella decora*.

The rhizophores have a length of 30 to 45 millimetres and a diameter of  $\frac{3}{4}$  to 1 millimetre. The cells of the epidermis are much smaller than those of the hypodermis; the cell-walls of both are thick. The cortex is composed of 10 to 15 rows of cells. In comparison with the thickness of the aerial root, the vascular bundle is not as well developed as in other species. The protoxylem is composed of three to four tracheides and the metaxylem also of three to four. The phloem is, in comparison, better developed. The construction of the very thin roots is very much like that of the previous species.

*Selaginella Douglasi*.

The length of the rhizophores varies from 20 to about 28 millimetres; its diameter from  $\frac{1}{3}$  to  $\frac{1}{2}$  millimetre. The cells of epidermis, hypodermis, and mostly those of the cortex, are very thick-walled in plants which were developed in their native country (Oregon State, U.S.A.), where they have the habit of xerophytes growing upon stems of trees. Individuals which were grown in moist soil, and in the damp environment of a greenhouse, show considerably less thickened walls in the above-mentioned tissues. The xylem is well developed, and is composed of about five tracheides belonging to the protoxylem, and ten to sixteen belonging to the metaxylem. The phloem which surrounds the xylem is much like that of the stem.

*Selaginella molliceps*.

This species forms strong well-developed rhizophores, especially when twigs are growing close to one another. They attain a length of 6 to 15 millimetres and a diameter of  $\frac{1}{3}$  to  $\frac{1}{2}$  millimetre. The epidermis is composed of rather large cells, although somewhat smaller than those of the cortex. The cuticle is thin, even that of older rhizophores. The terrestrial root does not show marked differences, except that as usual the epidermis is followed by two thin-walled layers of cells. The development of the vascular bundle is like that of *S. decora*.

*Selaginella Bakeriana*.

The rhizophores somewhat resemble in anatomical construction that of *S. rubella*. They reach a length of 6 to 12 millimetres and a diameter of  $\frac{1}{3}$  to  $\frac{1}{2}$  millimetre. The cells of the epidermis and hypodermis remain

thin-walled for a long time, only being thick in the older rhizophores and in two or three layers of the outer cortex (Fig. V, 1 and 2). The secondary layers in the walls of younger rhizophores are not formed as soon as those in several other species. The vascular bundle is like that of *S. rubella*; there are, however, not as many tracheides in the metaxylem.

The terrestrial root has, with the exception of the epidermis and the two following rows of cells, which are large, the same construction as the stem. The cells of the hypodermis are slightly thick-walled.

*Selaginella viticulosa.*

This species develops aerial roots very close to the base of the stem; they attain a length of 3 to 8 millimetres and a diameter of  $\frac{1}{4}$  to  $\frac{1}{3}$  millimetre; consequently very little is exposed to the air.

The cells of the epidermis, hypodermis, and cortex are almost of the same size, and those of the first two tissues are thick-walled when they become older. The epidermis has no developed cuticle. All the walls of the epidermis and hypodermis are encrusted with a red pigment; the walls of the cortex cells are colourless. The cortex is composed of eight to ten rows of cells. The vascular bundle is comparatively little developed; its xylem is composed of three to four tracheides belonging to the protoxylem and four to five wide ones belonging to the metaxylem.

Of the terrestrial root, which shows very much the same characteristics, it may be mentioned that there also the hypodermis is red-coloured for some length, but this colour disappears when the roots penetrate deeper into the soil.

*Selaginella serpens.*

The aerial roots attain a length of 6 to 12 millimetres and a diameter of  $\frac{1}{4}$  to  $\frac{1}{3}$  millimetre. The epidermis and hypodermis are composed of cells whose walls are slightly thickened; the cortex is composed of three to five layers of thin-walled cells. The endodermis and pericycle are composed of small cells which are frequently difficult to distinguish from those of the outer cortex. The xylem is composed of only seven to eight tracheides, of which two to three belong to the protoxylem.

The terrestrial roots (Fig. X) are very thin; the epidermis and the two following rows of cells are rather large; root-hairs are formed abundantly and are relatively long; the hypodermis is composed of about three rows of cells, which contain some secondary walls; the cortex is formed by about three to four cell-layers, of which all the cells are much smaller than those of the hypodermis, and especially those of the epidermis. The vascular bundle is in all instances very small; it is composed of a few sieve-tubes; the xylem is formed by, as a rule, only three tracheides, which seem to belong to the protoxylem.

*Selaginella formosa*.

The aerial roots have a length of 40 to 60 millimetres and a diameter of  $\frac{1}{2}$  to  $\frac{3}{4}$  millimetre. The epidermis is relatively large-celled, also the hypodermis. The cuticle is well pronounced; the cells of the hypodermis are not as thick as is the case with other species. The cells of the cortex are composed of seven to eight rows of cells and are larger than those of the

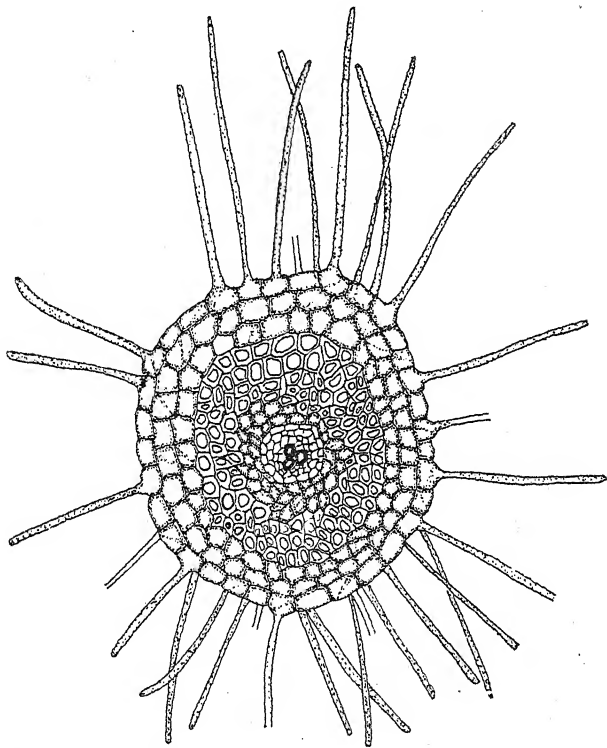


FIG. X. Section of root of *S. serpens*.

hypodermis. The endodermis, as a rule, can be well distinguished from the other tissues. The xylem possesses eight tracheides in the protoxylem and five to eight tracheides in the metaxylem.

The terrestrial roots show, with the exception of the first three cell-layers, the same anatomical construction as that of the rhizophore; although the cortex is not composed of as many layers of cells, the hypodermis on the other hand remains the same. The vascular bundle is almost the same: there are only a few tracheides less.

*Selaginella apus*.

The rhizophore is very thin and fibre-like; its length amounts to 5 to 12 millimetres and its diameter  $\frac{1}{4}$  to  $\frac{1}{3}$  millimetre. The cells of the

epidermis and hypodermis are very large, almost as large as those of the cortex cells. Cells of the epidermis and hypodermis only contain slightly secondary layers. The vascular bundle is small, although its elements are all represented; the xylem is only composed of five to six tracheides, of which two to three belong to the protoxylem. There is little difference in the construction of terrestrial roots.

*Selaginella Galeotti.*

The rhizophores attain a length of 80 to 120 millimetres and a diameter of  $\frac{1}{2}$  to  $\frac{3}{4}$  millimetre. The epidermis cells are small; those of the hypodermis are much larger and as big as those of the cortex. The cell-walls of epidermis are very thick; very little space has been left in those of the older rhizophores. In many cells of the cortex a red pigment is developed in the vacuoles, or at least those which developed towards the side of the approaching light. The cortex is formed by twelve to twenty layers of cells. The vascular bundle, especially the xylem, is not as much developed as one would expect; there are four to five tracheides in the protoxylem and about five in the metaxylem. The terrestrial root, which is much thinner than the aerial root, has a less developed cortex, which is formed by three to four layers of cells; although otherwise it shows much the same development as the aerial root.

*Selaginella patula.*

The aerial root reaches a length of 10 to 18 millimetres and a diameter of  $\frac{1}{2}$  to  $\frac{3}{4}$  millimetre. The cells of the epidermis, hypodermis, and cortex are practically of the same size; only those of the inner cortex are larger. The cuticle is very little pronounced, although the other walls of the epidermis and those of the hypodermis are furnished with secondary layers. The vascular bundle does not differ much from that of *S. molliceps*. The terrestrial root also does not show much difference, except that part which is directly in touch with the soil.

*Selaginella Martensi.*

The rhizophore becomes 30 to 60 millimetres in length and  $\frac{3}{4}$  to 1 millimetre in diameter. The epidermis has a thick cuticle on plants which have been rather exposed, and as thin as an ordinary primary wall when developed in a shady and very moist environment. The other walls of the epidermis and those of the hypodermis are thickened in the same degree. The cortex is composed of twelve to fifteen usually thin-walled rows of cells. Towards the vascular bundle they become smaller. The endodermis and pericycle are very distinct. The elements of the phloem are well represented and hardly differ from those of a stem. The xylem is formed by four to five vessels of the protoxylem and ten to fourteen belonging to the metaxylem.

The terrestrial root has a large-celled epidermis, which forms relatively long root-hairs; it is followed by two other thin-walled layers, whose cells are of the same size; this tissue is succeeded by three rows of cells belonging to the hypodermis, which as usual are thick-walled. There is no difference in the vascular bundle in comparison with that of the aerial root, only that its elements are relatively fewer the thinner the root in the soil becomes.

*Selaginella atrovirens.*

The rhizophore has a length of 35 to 65 millimetres and a diameter of  $\frac{3}{4}$  to 1 millimetre. All the cells of the epidermis, hypodermis, and sometimes of the outer cortex are furnished with secondary walls, although these are not very thick. The cortex is composed of eight to twelve rows of cells, which are larger than the hypodermis cells. The vascular bundle is as a rule surrounded by a distinct endodermis and pericycle. The xylem takes considerable room in the vascular bundle, and is more developed than the phloem. The metaxylem is composed of twenty to twenty-eight tracheides; the protoxylem sometimes contains eight to fifteen of such vessels, and is strongly developed in the primary rhizophore.

The terrestrial roots are thicker than those of most other species, and attain a thickness of  $\frac{1}{2}$  to  $\frac{3}{4}$  millimetre. The root-hairs are not very abundant. The epidermis, as a rule, is succeeded by two other thin-walled layers; the hypodermis is composed of cells which are of rather considerable thickness, especially in those which grow close to the surface; those which have developed considerably deeper in the ground are in possession of a hypodermis which has not as many secondary layers in its walls.

*Selaginella grandis.*

The aerial root reaches a length of 8 to 12 millimetres; in one instance I found one of 25 millimetres; its diameter varies from  $\frac{1}{2}$  to 1 millimetre. There are two different types of rhizophores, one of which has developed a root-cap and root-hairs; the other has no root-hairs and no root-cap. The primary rhizophore, which is in direct connexion with the stem, very rarely develops root-hairs, and never a root-cap, as far as I was able to observe. While the rhizophore with root-cap develops *all* its epidermis cells into root-hairs, the other one without root-cap occasionally forms a root-hair from an epidermis cell, leaving the other cells of epidermis to their normal development.

The aerial root without root-cap is composed of small cells; those of the epidermis, hypodermis, and cortex are of the same size. The two former tissues are thick-walled; the cortex is composed of ten to fifteen rows of cells. The vascular bundle is very small, although it is very strongly developed in the stem. The endodermis cannot be clearly distin-

guished from the small cells of the inner cortex. The xylem is composed of six to nine tracheides; those of the protoxylem are very narrow. Aerial roots which are developed in a very moist environment always develop root-hairs and root-cap. As has been stated, every epidermis cell changes into a root-hair; these hairs are narrow and long. The epidermis is followed by only one thin-celled layer, which is succeeded by a thick-walled hypodermis. There are less rows of cells forming the cortex than in a root without root-hairs (Fig. III, 3 to 9).

Terrestrial roots are the same as the latter kind of roots.

*Selaginella cuspidata.*

Rhizophores are only developed towards the base of the stem, and only when growing in a very damp environment; plants which grow in their

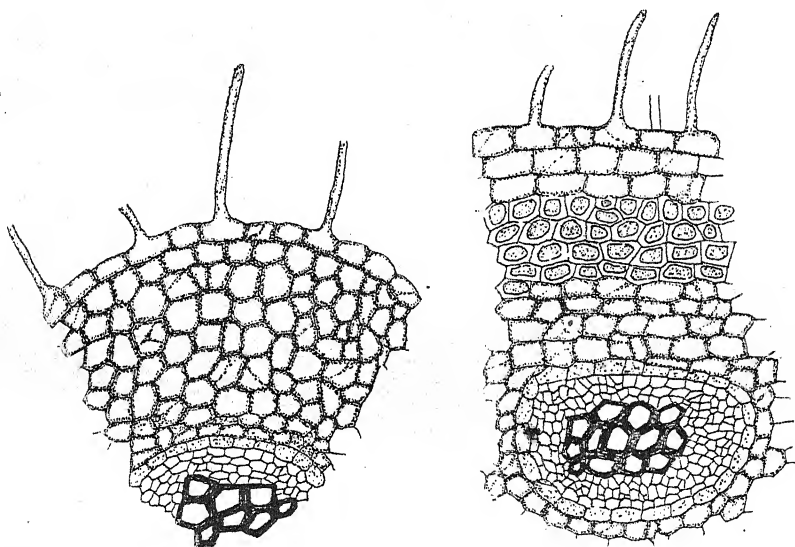


FIG. XI. Section of root of *S. Bakeriana*.

FIG. XII. Cross-section of root of *S. rupestris*.

native environment do not develop such roots, which is also the case with other xerophytic species, e.g. *S. pilifera* and *S. lepidophylla*. They attain a length of 5 to 8 millimetres and a diameter of 1 to 1½ millimetres. These are very tough and velvety; the former quality is caused by its well-developed sclerenchyma, the latter by the presence of innumerable root-hairs. The writer was not able to observe aerial roots without root-hairs.

A section of the root (Fig. XIII, 1 and 2) shows the following: the epidermic cells are all transformed into root-hairs, after which follows one layer of thin-walled cells; this tissue is followed by layers of hypodermic and cortex cells, which are composed of eight to nine rows of cells. These are succeeded by six to eight rows of thin-walled cortex cells;



those approaching the vascular bundle are considerably smaller, which makes the endodermis less clearly visible. The phloem as well as the xylem

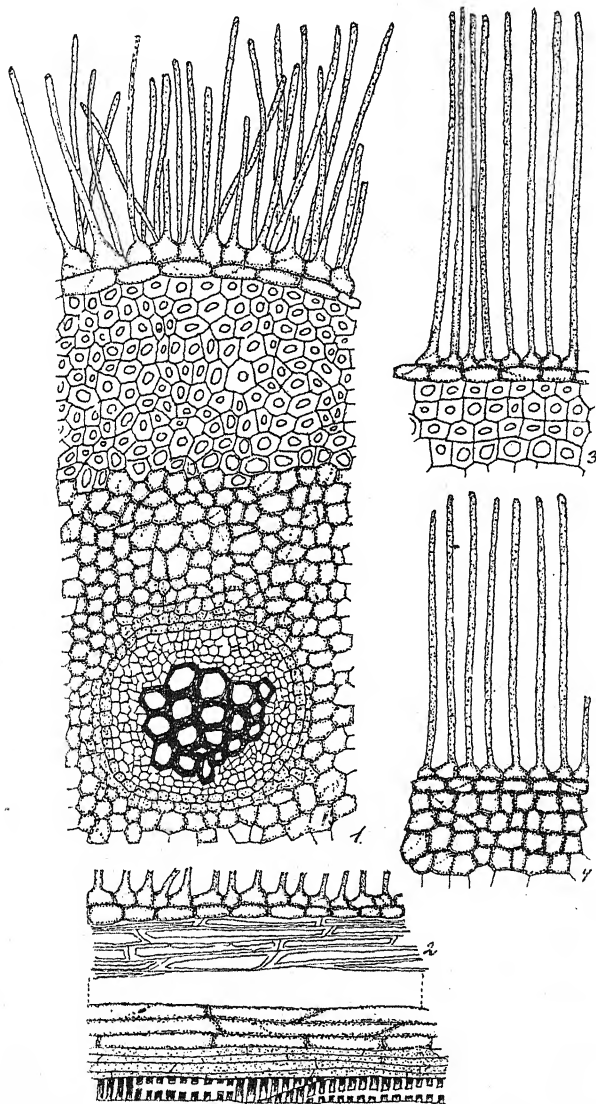


FIG. XIII. 1, 2, *S. cuspidata*: 1, cross-section of aerial root; 2, longitudinal section of same. 3, 4, *S. pilifera*: 3, old rhizophore; 4, young rhizophore.

is well developed. The protoxylem is composed of about five tracheides; the metaxylem of ten to fourteen.

The terrestrial roots are exactly like the former. Roots which are developed in a semi-arid desert region have as a rule a cortex which is

almost entirely thick-walled, with the exception of two or three rows of cells towards the endodermis.

Roots of *S. pilifera* and *S. lepidophylla* are very much the same; only the root-hairs are longer.

*Selaginella rupestris.*

The length of the aerial roots is 4 to 8 millimetres, and the thickness  $\frac{1}{4}$  to  $\frac{1}{3}$ , sometimes  $\frac{1}{2}$ , millimetre. The epidermis is thick-walled and furnished with a clear cuticle; the hypodermis also is composed of thick walls. The cortex is formed by six to eight rows of thin cells in plants which were developed in a moist environment, but are thick-walled in plants which were grown on exposed rocks. There is no difference in construction of the vascular bundle between that of a root and of a stem, which applies to all species of the homophyllous Selaginellas, e.g. *S. rupicola*, *S. densa*, *S. capensis*, and other species, a fact which is also to be noticed in the case of the root and stem of *Lycopodium*.

The terrestrial root shows a large and thin-celled epidermis, which forms short and rather wide root-hairs. These are succeeded by two rows of thin-walled cells of the same size; after which a hypodermis is to be noticed attaining a thickness of three or four rows of cells. The cortex is usually composed of four rows of cells. The vascular bundle is of the same type as that of the aerial root and stem (Fig. XII).

#### SUMMARY.

The species of *Selaginella* are remnants of older periods in which also other related genera reached their highest pitch of development. They belong to the primitive vascular plants, and therefore morphologically are of much importance as far as the origin of the plant organs is concerned.

Some of the present living allies of *Selaginella* are in possession of true roots, although anatomically these do not differ very much from that of the stem (e.g. *Lycopodium*); some do not have true roots, such as species of the Psilotaceae.

Generally the root is simple in construction and differs from the stem, the former lacking the lacunae and trabeculae of the latter.

There is no important anatomical difference between aerial and terrestrial roots. The difference of the outer tissue is due to environmental circumstances.

Physiologically both kinds of roots have the same characteristics in the same degree; both are negative heliotropic.

Roots with root-cap always have root-hairs, those without root-cap very rarely have root-hairs.

Of all species, the root originates with regard to the stem exogenously; they branch monopodially.

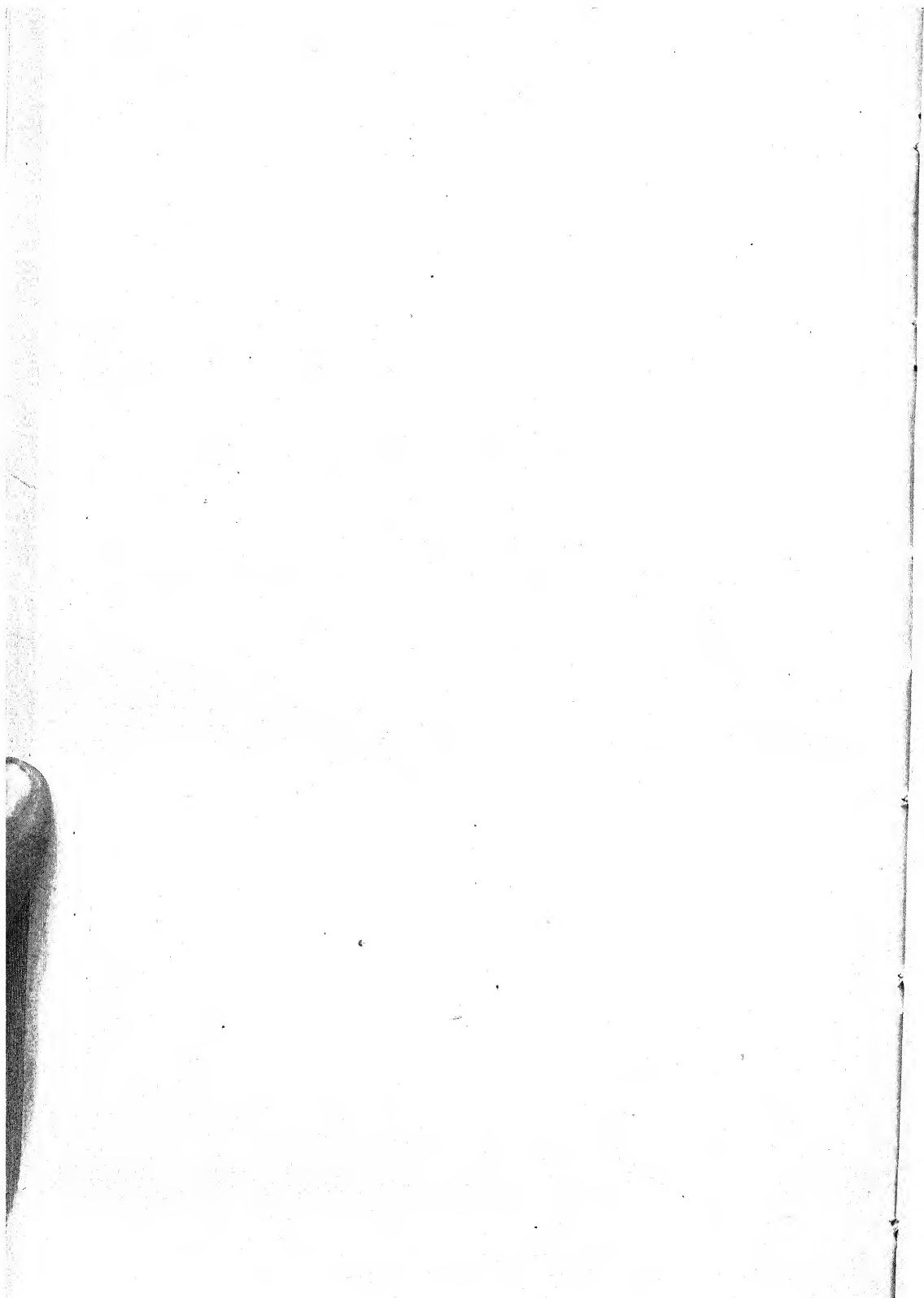
Some species form, when bruised, small stems instead of rhizophores; these suggested to many investigators that the latter are leafless stems. The anatomy shows that these branches have the same construction as a stem and are positive heliotropic, whereas the so-called rhizophores have the construction of a root and are negative heliotropic.

The vascular system is monarch; the endodermis and pericycle are always present. The phloem shows the same arrangements as in the stem, although its elements are less abundant. The xylem is composed of one group of protoxylem and usually a well-developed metaxylem.

The thick-walled tissue which follows the three layers of thin-walled cells of the periphery of the terrestrial roots apparently belongs to the hypodermis.

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## A New Species of *Spirogyra*.

BY

WILLIAM J. HODGETTS, M.Sc.

With Plate XXII and five Figures in the Text.

DURING May, 1919, a number of species of *Spirogyra* (including *S. calospora*, *orbicularis*, *nitida*, *longata*, *catenaeformis*, *inflata*, &c.) were observed in conjugation in a pond, which has been visited periodically for some time, at King's Norton, near Birmingham, and amongst these and other filamentous Algae occurred a *Spirogyra*, which not only appears to be a new species, but which presents several interesting features hitherto undescribed for the genus.

The vegetative cells of this species are 29-40  $\mu$  wide, while their length is 8-16 (sometimes as much as 22) times as great as the diameter. The filaments always show a conspicuous gelatinous sheath, which, even without the aid of stains, usually exhibits a very well-marked fibrillar structure (cf. Text-fig. 4). On mounting filaments in Indian ink it is easily seen that this sheath consists of two layers, and that the outer layer shows no obvious structure, the fibrillae being confined to the inner layer, which is also somewhat thicker than the outer. These fibrillae—which, of course, occur in the sheaths of certain other filamentous Conjugates, as well as in those of some Desmids—have a remarkable resemblance to large rod-shaped Bacteria, and give the filaments quite a furry appearance—shown in Photo 1, Pl. XXII; if a hand-lens is used, however, the fibrillar structure will be obvious in this photograph. It was rare for the whole sheath to be apparently structureless.

The most curious feature, however, is the development between every contiguous pair of cells of an H-shaped piece of membrane, connecting the two cells together like a clamp (see Text-figs. 3, 4). This connecting-clamp is a thin cylindrical piece of cell-wall, 25-30  $\mu$  long, and furnished in the middle with a thin septum, so that the appearance in optical section is like the letter H. Although it gives the reaction for cellulose like the rest of the cell-wall, the longitudinal portion of the clamp-like connecting-piece is rather thinner than the main cell-wall, while its transverse septum is extremely thin. Each half of the connecting-piece fits tightly like a cap

over the end of its corresponding cell, which at this part is slightly narrowed to accommodate it, the actual diameter of the vegetative filament thus remaining fairly uniform throughout the entire length (compare Text-fig. 3).

The origin of this curious clamp-like organ—which, as far as the writer is aware, has never been described in any other species of *Spirogyra*—is as follows: The transverse septum (which is not replicate), separating two young daughter-cells, becomes rather thickened and biconvex in form, while at the same time the longitudinal cell-wall for a distance of about  $12-15\mu$  on each side of the transverse wall becomes thickened to nearly twice its normal thickness (Text-fig. 1). Two splits now arise in the thickened septum, one on each side of the original 'middle lamella' (Text-fig. 2), which itself persists as the septum of the H-shaped connecting-clamp. Each split extends from the middle outwards, but follows the contour of the end of its corresponding cell, so that although originally transverse it turns and becomes longitudinal and finally forms the clamp-connexion by turning abruptly outwards just where the thickening of the longitudinal wall ceases, as shown in Text-fig. 3. This figure (as well as Text-fig. 4) shows the slight outward curl of the edges of the connecting-piece—a character which was fairly constant.

The exact function of this connecting-piece is rather obscure, although it may be considered as a clamp-like organ tending to keep the cells of a filament together when excessive turgidity of the cells, or some other cause, is acting to produce the opposite result, i.e. the breaking up of the filament into its individual cells. It is clear that a cell must be pushed at least a distance equal to half the length of the connecting-clamp (i.e. a distance of about  $12$  to  $15\mu$ ) before it becomes quite free from its neighbour. Text-fig. 4 shows the ends of two disconnected cells, one of which has retained the connecting-piece as a sort of cap; a state of affairs exactly resembling that shown in the bottom right-hand corner of Photo 4, Pl. XXII. Photo 5 is especially interesting, since it shows a connecting-clamp—the septum of which is quite visible—which has just slipped off the end of a (curved) vegetative cell; and moreover the latter itself is seen to have partly slipped out of the clamp by which it is connected to the filament. Since it was rather rare to find the clamp-connexions lying free in the water, it seems that they are generally retained by one of the cells when the filaments break up.

The fibrillar structure of the external gelatinous sheath was generally denser round the connecting-clamps than elsewhere (see Photos 1-3, Pl. XXII), due probably to the fact that the clamps undergo no longitudinal growth, while the cells themselves often attain a relatively great length.

The cells show 5 (sometimes 4 or 6) narrow chloroplasts, each making a very lax spiral ( $\frac{1}{2}$ – $1\frac{1}{2}$  turns), while the pyrenoids are large, projecting considerably from the chloroplasts, and surrounded with large angular

starch-grains. Not infrequently certain individual cells of a filament are curiously coiled, making  $\frac{1}{2}$ –2 turns, although otherwise quite normal, and sometimes observed in conjugation. Photos 4, 6, and 7, Pl. XXII, show examples of these coiled cells. Coiling of the filaments of various filamentous Algae has been recorded by W. and G. S. West, the specimens

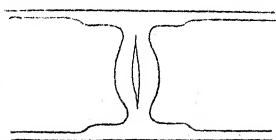


Fig. 1.

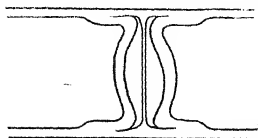


Fig. 2.

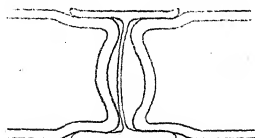
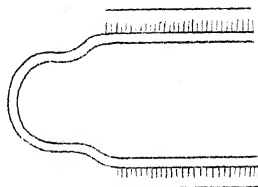
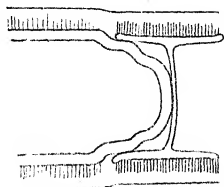
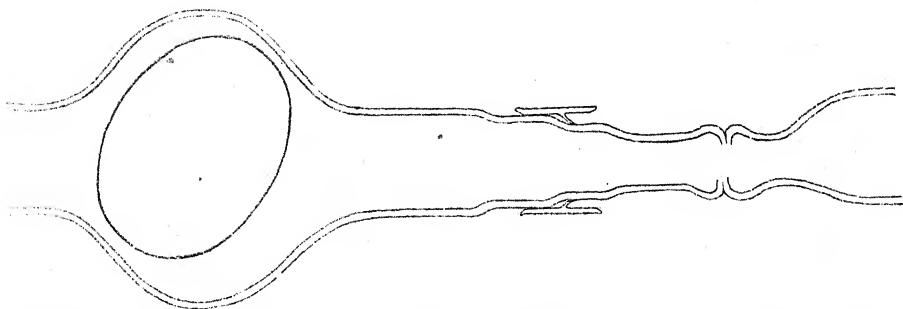


Fig. 3.

TEXT-FIGS. 1–3. *Spirogyra colligata*, sp. nov. Successive stages in the development of the H-shaped connecting-clamp between contiguous pairs of cells (the process is described in the text).  $\times 500$ . (Somewhat diagrammatic.)



TEXT-FIG. 4. *Spirogyra colligata*, sp. nov. Ends of two contiguous cells which have been pulled apart, showing the H-shaped clamp-connexion retained on the end of the left-hand cell. The gelatinous sheath with its fibrillar structure is shown (in the other figures it has been omitted).  $\times 500$ . (Somewhat diagrammatic.)



TEXT-FIG. 5. *Spirogyra colligata*, sp. nov. Terminal conjugation. Only the end of the empty male gametangium is shown.  $\times 400$ . (Somewhat diagrammatic.)

occurring in the plankton of lakes, and the coiling considered to be 'a limnetic character developed to augment the floating capacity of the filament'.<sup>1</sup> This view can hardly be applied to the coiled cells of the present Alga, since the filaments occurred, amongst other filamentous forms, at the shallow margin of a pond, and generally in less than 12 inches of water. Moreover, in the present case the coiling was generally limited

<sup>1</sup> W. and G. S. West, in Proc. Royal Soc., B., vol. lxxxi, 1909, p. 195. Coiled filaments have been recorded in species of *Mougeotia*, *Anabaena*, *Lyngbya*, and *Melosira*.



to a single cell of a filament, and in several cases it was clearly seen that these curved cells were coiled *round another filament of the same species*. This can hardly be considered accidental, and it appears that this coiling may be a contact phenomenon serving to grapple the filaments together, and thus aiding scalariform conjugation.

Three modes of conjugation were observed in this species: (1) scalariform conjugation (Photos 1 and 7, Pl. XXII), which was frequent; (2) lateral conjugation—the conjugation-tubes either growing round the clamp-connexion, or else piercing it laterally (Photo 2, Pl. XXII); and (3) *terminal* conjugation. The last, and most interesting, method is brought about by the growing out of one extremity of a cell, in the form of a conjugation-tube, *through* the septum of the H-shaped clamp, to meet, and eventually fuse with, another terminal conjugation-tube put out by the adjacent cell of the same filament. Photos 1, 3, 5 to 8, Pl. XXII, show examples of terminal and subterminal conjugation, these being the methods which were most frequently observed. In both it will be seen that the two conjugating-cells are pushed some distance apart by the growing out of the terminal or subterminal conjugation-tubes; and the H-shaped connecting-clamp, through the septum of which one of the conjugation-tubes has to pass, is either pushed off both the cells and remains transfixed somewhere on the conjugation-tube—shown very well by Photo 8, Pl. XXII—or else it persists on the end of one of the cells, as shown in Photos 6, 7, and in Text-fig. 5.

True lateral conjugation, such as is shown in Photo 2, Pl. XXII, was rather rarely met with, but all stages intermediate between exactly terminal conjugation (as shown in Photo 8) and typical lateral conjugation (Photo 2) were observed, and indeed were very frequent. Subterminal conjugation is illustrated in the upper part of Photo 1, Pl. XXII, and also by Photo 5. It must therefore be understood that the terms 'lateral' and 'terminal' as applied to modes of conjugation in this species simply denote extreme cases of what amounts practically to one and the same method.

Photo 4 shows an example (on the left) of subterminal conjugation, but the central (immature) zygospore has been produced by a combination of the scalariform and terminal methods, the conjugation-tube put out from the female gametangium—which is the end-cell of a filament—being approximately terminal. This, however, is an abnormal mode of conjugation, and was observed only twice.

Terminal (or subterminal) conjugation does not appear to have been described as a normal method of conjugation in any other species of *Spirogyra*, as far as the present writer is aware. It should be noted that an attempt at this method of conjugation in any other species of the genus—whether provided with replicate end-walls or not—would probably fail owing to the two conjugating-cells being pushed apart before a firm union became established between the conjugation-tubes. In the present species,

however, on account of the H-shaped clamp, the cells are more firmly joined together—as stated above, one could be pushed a distance of 12–15  $\mu$ , or even more, before becoming actually free from its neighbour—and probably this it is which enables the two terminal conjugation-tubes to fuse together and effect a firm union.

The zygospores are lenticular in shape, being quite circular in front view but broadly oval in the side view (see Text-fig. 5). The wall of the mature zygospore is three-layered, as in other species, the outer and inner layers being thin, smooth, and colourless, while the middle layer is thick, brown in colour, and verruculose, presenting a wavy appearance when viewed in profile.

The female gametangium, as shown in the photographs on Pl. XXII, is swollen in the middle, often to a considerable extent and much more than is necessary to accommodate the zygospore. The only other species of the genus which have the female cells swollen in this way, and with which the species now being described might be confused on a superficial examination, are *Spirogyra pellucida*, (Hass.) Kütz.,<sup>1</sup> and *S. sphaerospora*, Hirn.<sup>2</sup> The present species differs from *S. pellucida* in its narrower cells, in the presence of the H-shaped clamps between the cells, and in the possession of (generally) 5 chloroplasts. *S. sphaerospora* has a single chloroplast, smooth-walled zygospores which are 'vollkommen kugelig', and no H-shaped pieces between the cells.

The writer considers this new species to be one of the most highly evolved, if not the highest, of all the known species of *Spirogyra*. The curious H-shaped connexions—not described for any other species—which tightly clamp the cells together, as well as the variety of modes of conjugation, seem to support this view. At any rate the species is of particular interest, since it shows that the genus *Spirogyra* is much more plastic, both in vegetative structure and in methods of conjugation, than has hitherto been generally supposed.

The species has been called *S. colligata*, on account of the cells being 'bound together' by the H-shaped connecting-pieces; and the following is a diagnosis of it:

*SPIROGYRA COLLIGATA*, sp. nov.

Sp. lubrica, pallide viridis, cellulis vegetativis plerumque rectis, at interdum spiraliter curvatis, diametro 8–16(–22)-plo longioribus, extremitatibus non replicatis (sed ut plurimum plus minusve alte incurvis); cellulis binis contiguis confibulis H-formibus, e membrana cellularum externa efformatis, arcte colligatis; chromatophoris 5 (interdum 4 aut 6), angustis,

<sup>1</sup> See West, G. S.: Journ. Bot., 1899, p. 109.

<sup>2</sup> Hirn: Acta Societatis pro Fauna et Flora Fennica, xi, No. 10, p. 10. I am indebted to the late Professor G. S. West for information concerning this species.

pyrenoidibus magnis instructis, anfractibus laxissimus  $\frac{1}{2}$ – $1\frac{1}{2}$  (–2); coniugatione scalariformi vel laterali vel terminali; cellulis fructiferis parte mediana valde tumidis; zygosporis lentiformibus, a fronte visis orbicularibus, a latere late ovalibus, cellulas fructiferas non complentibus, membranae strato mediano verruculoso.

Crass. cell. veget. 29–40  $\mu$ ;

„ „ fruct. circa 90–100  $\mu$ ;

Diam. zygospor. 50–80 (–90)  $\mu$ .

*Hab.* in stagno King's Norton, Worcestershire (May 1919).

My best thanks are due to Mr. W. B. Grove, M.A., for his kind interest and help during the preparation of this paper.

## DESCRIPTION OF PHOTO-MICROGRAPHS OF *SPIROGYRA* *COLLIGATA*, SP. NOV., IN PLATE XXII.

Illustrating Mr. William J. Hodgetts's paper on a New Species of *Spirogyra*.

(All these figures are from untouched photographs.)

Photo 1. The upper zygosporis has been formed by semi-terminal conjugation—the empty male gametangium on the left is only partly shown. The lower zygosporis is the result of scalariform conjugation. The furry appearance of the male cell is due to the pronounced fibrillar structure (seen better if the photograph is examined with a hand-lens) of the gelatinous sheath. Note the H-shaped clamp-connexion which has persisted on the end of the (lower) female gametangium.  $\times 90$ .

Photo 2. True lateral conjugation, the empty male gametangium being on the right.  $\times 90$ .

Photo 3. Semi-terminal conjugation.  $\times 82$ .

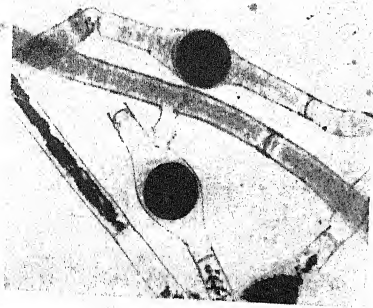
Photo 4. The zygosporis on the left has been produced by terminal conjugation (the male cell is only partly shown on the left). The immature central zygote has been produced by an abnormal method compounded of the 'terminal' and 'scalariform' methods, the female conjugation-tube being terminal. At the bottom right-hand corner of the photo the ends of two disjointed cells, with the H-shaped clamp (loosely fitting on end of upper cell) which formerly joined the cells, will be seen (cf. Text-fig. 4). Two examples of coiled cells are also shown.  $\times 90$ .

Photo 5. Semi-terminal conjugation. The (curved) end-cell is attached very loosely to the filament, and an H-shaped clamp-connexion, which has just slipped from its extremity, is shown.  $\times 110$ .

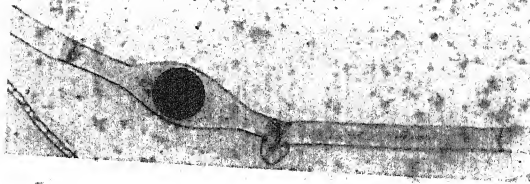
Photo 6. Terminal conjugation and a coiled vegetative cell.  $\times 85$ .

Photo 7. Scalariform and two examples of subterminal conjugation are illustrated. Two coiled cells are also present.  $\times 90$ .

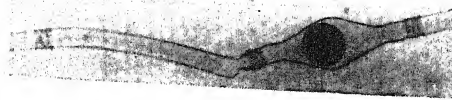
Photo 8. Terminal conjugation (the empty male gametangium is only partly shown). The H-shaped clamp has been pushed off the ends of the conjugating-cells, and is seen transfixed on the conjugation-tube between the two gametangia.  $\times 90$ .



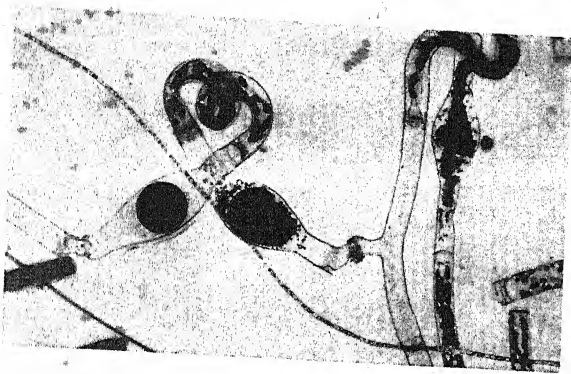
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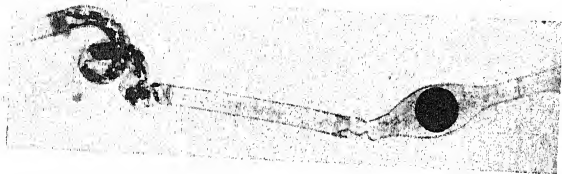
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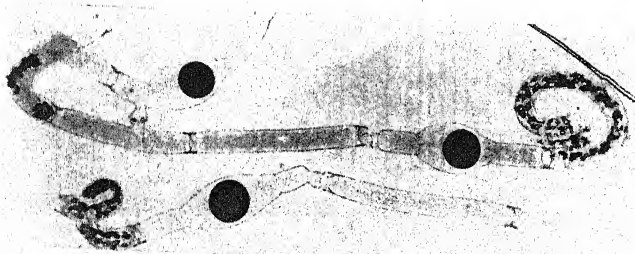
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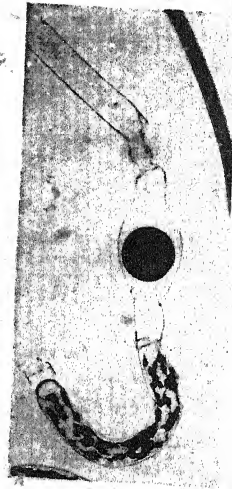
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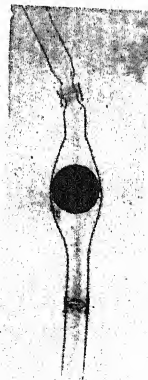
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W.J.H. photo.

HODGETTS-SPIROGYRA

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# The Anatomy of *Rhododendron ponticum*, L., and of *Ilex Aquifolium*, L., in Reference to Specific Conductivity.

BY

MAUD F. RIVETT, B.Sc.

With seventeen Diagrams in the Text.

THIS paper is the result of an investigation into the anatomical characters of *Rhododendron ponticum* and *Ilex Aquifolium* with the object of ascertaining the anatomical basis for variations in the water-conductivity of the wood.

In experimenting on the water-conducting efficiency of the wood in shrubs and trees, Professor Farmer has shown that not only are there great differences in the water-carrying powers of deciduous and evergreen trees and shrubs, but there may be also variation among the stems and branches of the same species when taken from plants growing under different conditions and in different localities.<sup>1</sup>

The term 'specific conductivity' is used to express the volume of water transmitted by a segment of stem, 15 cm. in length, per 1 sq. cm. of wood as seen in transverse section. This volume, given in cubic centimetres for the period of 15 minutes, varied for 28 holly twigs from 6.8 up to 12.2, with an average of 8.7: for *Rhododendron ponticum* it varied for 50 stems from 10 to 25 for the majority, though a few were much lower than 10 and a few much higher than 25. It is stated that, in spite of its evergreen habit, such variation is to be expected in a plant which is so freely branched and has so wide differences in the development of its branches that they vary almost from abortion to great luxuriance.

## RHODODENDRON PONTICUM.

*General Morphology.* *Rhododendron ponticum*, L., is a native of the Caucasus, but is cultivated in the open air in England and on the Continent, and develops well under suitable soil conditions. In a sandy soil the young plants will make shoots of 50 cm. in length during one season, but afterwards growth is slower, and older bushes rarely make annual shoots above

<sup>1</sup> J. B. Farmer: on the Quantitative Differences in the water-conductivity of the Wood in Trees and Shrubs, Parts I and II. Proc. Roy. Soc., B., vol. xc, 1918.



35 cm. in length. Though often described as xerophytic in character, it has been observed that they are more liable to suffer from drought than the laurel and the *Aucuba*, with which they are sometimes associated in decorative shrubberies. The year's growth of a shoot is very characteristic. The winter-bud is enclosed by scales which are morphologically entire foliage leaves reduced in size: in the spring the internodes between the scales elongate considerably and the scales themselves enlarge slightly, so that they assume a form transitional between scales and true foliage leaves. They are rather delicate in texture and fall off after a short time, leaving a narrow scar, which half encircles the stem. In the early part of the summer the true, persistent foliage leaves are developed. They are often very large, 12 to 15 cm. in length, and xerophytic in character. The petioles are thick and terete, leaving a circular scar where the leaves fall. They are not separated by well-marked internodes, but are crowded together in a rosette-like form. The length of the internodes is short but extremely irregular. The position of the leaves in the full-grown shoots is not affected by the light, so that the shoots retain their radial form, but there is a certain two-sidedness apparent, due to the greater growth of the leaves on the abaxial side of the axillary shoots, and on that side of the leading shoots away from the centre of the bush. The shoots are not fully matured at the end of the first season's growth, being softer and more flexible than they become later. In the axils of the lowest deciduous transitional leaves, buds are rarely developed, though they may appear in the upper ones. In the axils of the foliage leaves are large protected buds, which may remain dormant some time before they develop. The larger buds are on the abaxial side of the shoots. When the shoot terminates in a flower-bud, all the large axillary buds, some of which may remain dormant in a vegetative shoot, develop into an apparent whorl of lateral branches. These grow about 25 cm. in length in their first season and may terminate either vegetatively or in a flower-bud. In the former case the shoot continues its growth monopodially for the next season with a possible whorl of smaller laterals immediately below it.

*Material.* For this investigation the material consisted of mature shoots of one season's growth. These were in groups as they had been borne on the previous year's shoot, which was cut from the shrub near its apex. The groups are lettered and figured in Diagram I. The A group consisted of A 1, a main stem borne monopodically, and A 2, A 3, A 4, all lateral shoots. In the B group, B 1 is monopodial, B 2 and B 3 axillary. In the C and D groups, C 1 and D 1 are monopodial and the others axillary. In the E group there is no leading monopodial shoot, as the terminal bud had either been replaced by a flower or had aborted, but there are five axillary shoots, E 2 to E 6, of which E 2 assumes the form of a leading shoot. The groups were cut early in February 1919. The persistent leaves



were removed and lengths of 15 cm. were cut from each shoot and the specific conductivity registered experimentally. Subsequently the lengths were wired together and preserved in 70 per cent. alcohol.

In February the shoots are in their winter condition, showing the lower leafless part, marked with the scars of the fallen scale-leaves and a short

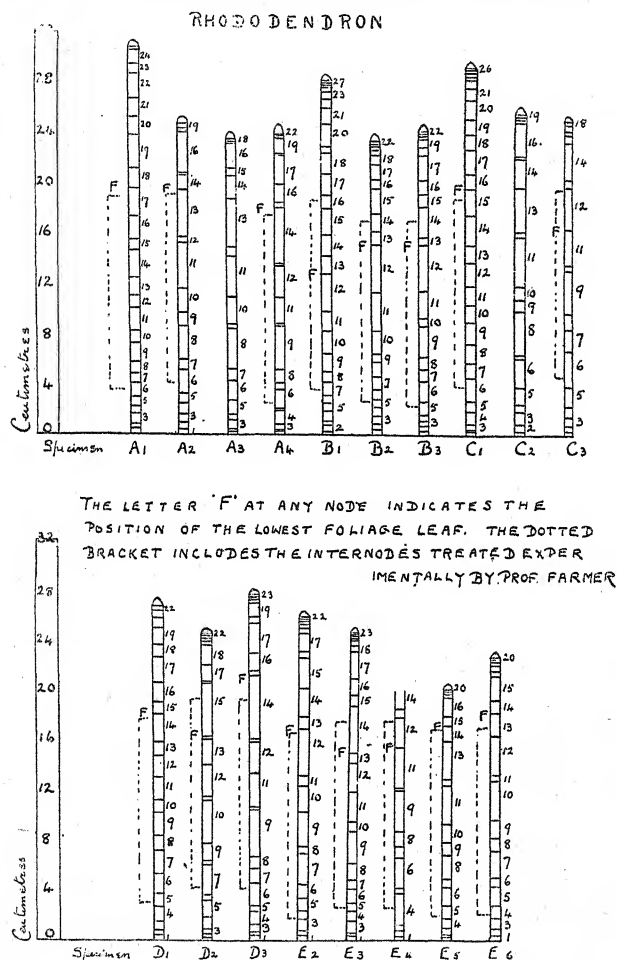


DIAGRAM I.

upper leafy part terminated by a dormant bud. In the axils of the foliage leaves the buds which are to produce next year's lateral branches are large and well developed. In general the shoots are between 20 and 30 cm. long, the longest in each group being the leading monopodial shoot. The details of each can be ascertained at a glance from Diagram I.

*General Anatomy.* In transverse section the stem shows narrow bark and cortex, a zone of wood which is wide at the base of the stem and

narrows upwards, and a wide pith which increases relatively towards the apex. It is hard and firm at the base, but becomes soft and flexible in the leafy region.

The wood, in transverse section, is seen to consist of dead lignified elements interspersed with living cells, and divided radially into narrow sectors by the medullary rays. The lignified elements include small thick-walled fibres and wide water-conducting elements. The first-formed wood (protoxylem) abutting on the pith consists of very small water-conducting elements, with thick walls, sometimes in process of disintegration. The water-conducting elements of the metaxylem are larger and occur in irregular groups just behind the protoxylem. The paucity of fibres in the primary xylem is very evident. At the period of growth at which it is functional, the supporting material of the stem is provided by a peripheral ring of hard fibres in the pericycle which are developed very early. Thus the first-formed wood is almost entirely a response on the part of the plant to the necessities of water-conduction. As the stem grows older, the protoxylem becomes squashed and obliterated, and elements of the metaxylem also lose their function, forming brownish patches. The secondary xylem occupies the main part of the wood cylinder. Peripherally the conducting elements are small and separated by wide patches of fibres, as is characteristic of autumn-formed wood, but for the inner two-thirds of the wood the numerous wide lumina of the water-conducting elements are very conspicuous. The lumina are very variable in shape, though many are elongated radially. The walls, though lignified, are comparatively thin and delicate, compared with those of the fibres and living cells. In transverse section it can be seen that the water-conducting elements communicate with living cells by bordered pits, with considerable thickening of the middle lamella. There are also shallower, less obviously bordered pits between adjacent conducting elements. The scattered living cells are more or less square in section, but where the living cells form uniseriate medullary rays they are wider radially than tangentially. Most of the rays are uniseriate, except in the neighbourhood of a leaf-insertion, where wider patches of living cells and numerous transitional tracheidal elements are to be found. All the living cells in the wood have rather thick walls and stain lightly with safranin. They have living protoplasm and a nucleus, but contain little starch at this season of the year.

In longitudinal section the water-conducting elements of the protoxylem are found to be elongated vessels with spiral thickenings. They reach a length of  $\frac{1}{4}$  cm., as can be more easily seen from macerated material. The segments forming the vessel are joined end to end without obvious remains of cross-walls, the perforations being large and entire. These elements of the protoxylem contrast strongly with the water-conducting elements of the secondary xylem, which are made up of segments about

fifteen times as long as broad, with their end-walls lying obliquely in the radial plane, inclined at an angle of about  $30^{\circ}$  to the longitudinal axis. The lateral walls show bordered pits when they are in contact with living cells and are strengthened by reticulate thickenings. The end-walls, which appear like bulging continuations of the lateral walls, are pierced by scalariform perforations, separated by scalariform bars which may number 17 or 18. The segments are in open continuity by means of the perforations, and thus form true vessels. Their length cannot be determined accurately by longitudinal section, since they run through several centimetres; nor by maceration, as the segments separate very easily; but reliable data can be obtained by injection with fine Indian ink. The shoots are soaked in water and the air removed by means of a suction pump: they are cut off (under water) as close to the base as possible and their basal ends immersed in a vessel of Indian ink: this is connected with a mercury pressure tube, which on elevation above the shoot will give a pressure of an atmosphere. The Indian ink is thus forced through the stem, the pressure being continued for 24 hours. When the shoot is removed, it is sectioned from the apex downwards, at intervals of 1 cm. The greatest height from the base at which the Indian ink is found in the wood indicates the length of the longest vessel. This maximum figure is indicative of the vessel-length, since the vessels injected do not show a continuous core of Indian ink, but a series of short interrupted columns, and many of those which appear to be empty in the basal region are found to be injected in the higher levels. The vessel-length shows considerable diversity in different shoots, as can be seen from the following table:

	Distance of Section from base in cm.	Number of Vessels injected.
Shoot 1	11	5
" 2	11	5
" 3	6	3
" 4	9	3
" 5	16	3
" 6	12	3

Thus the lowest figure (6 cm.) and the highest (16 cm.) represent lengths of stem in different shoots in which the vessels are continuous.

These long vessels with their short segments and small scalariform perforations form the characteristic tissue of the wood, and no unperforated tracheides are found associated with them in the wood cylinder. But these latter elements are numerous at the periphery of the wood in the neighbourhood of the insertion of the petioles: they are of varying form, often short, wide, and irregular, and serve to connect the petiolar wood strands with the long vessels of the wood cylinder.

The two-sidedness of the shoot, which is evident in the growth of the leaves, also shows itself in the anatomy. The cylinder of wood is widest on the abaxial and narrowest on the adaxial side of the stem. On the abaxial

side there is also a higher proportion of vessels to fibres than there is on the narrow side.

#### ILEX AQUIFOLIUM.

*General Morphology.* The Holly (*Ilex Aquifolium*) is a fairly rapid grower, making shoots up to 30 cm. long in a single season. The year's growth is simple, such as is found in most evergreen trees. There is a terminal winter-bud, bearing 5 or 6 spirally arranged scales. When the young shoot elongates in the spring, the scales fall off, but two or three transitional leaves are borne between the first elongated internodes, before the true foliage leaves appear: of these, fifteen may be borne in a single season. The internodes show the usual gradations in a year's growth—i.e. the longest are in the middle and the shortest towards base and apex. The transitional scale-foliage leaves fall during the first season, leaving a narrow encircling scar similar to that of the bud-scales. The foliage leaves may persist for four or five years, but usually some of the lower ones in each year's growth fall off in their second and third year. Buds are found in the axils of the scale leaves and the foliage leaves, but it is only in the upper regions of a year's growth that they normally develop into lateral branches. The shoots are soft and immature at the end of the first season, but they harden and increase in girth in successive years. The growth from year to year is monopodial, as it is the terminal winter bud which develops at the beginning of each season.

*Material.* For this investigation the material consisted of straight twigs of three, four, or five years' growth, cut in July 1919. The twigs are figured and lettered in Diagram II. From this it can be seen that the twig F, of total length 52 cm., consisted of three successive annual shoots—a 1916 shoot of 15 internodes, 22 cm. long: a 1917 shoot of 13 internodes, 19.6 cm. long: a 1918 shoot of 14 internodes, 10.4 cm. long—and an abortive 1919 shoot. Thus the twig exhibits four annual rings at the base, three in the middle, and two at the upper end. The scars of the winter-bud scales are taken as indicating nodes and are reckoned in the total internodes numbered from base to apex. The shoot G, of total length 52 cm., consisted of four successive annual shoots: the oldest, dating from 1915, shows five annual rings: the topmost, dating from 1918, shows two annual rings, there being again no one-year-old shoot, owing to abortion or accident in the early spring of 1919. The shoot H, of total length 63 cm., shows four successive annual shoots, including the uppermost still active in July 1919. The shoot K, showing five successive years' growth, is the longest and stoutest of the set and reaches a length of 77 cm. The shoot L shows great length, 58 cm.—considering that it is made up of three annual shoots only. The internodes are particularly long, straight, and well developed. The shoot M, of four years' growth, reaches a length of 73 cm.

Further details of these shoots can be seen at a glance from Diagram II, where they are drawn to scale to show the spacing of the nodes and internodes. It should be particularly noticed that the shoots F and G are truncated.

### General Anatomy.

In the transverse section of the stem there is a narrow bark and cortex, except in the first year's growth, where it may occupy about a third of the diameter. The pith appears relatively small at the base, but remains much the same actual size throughout a considerable length, being little affected by the tapering of the stem, so that it appears relatively large at the apex. The wood is very hard and consists of dead, lignified elements, separated radially by narrow medullary rays, which vary from one to four cells in thickness. The protoxylem elements are few in number and of narrow diameter. They are all water-conducting elements, the supporting tissue of the very young shoot being provided by a ring of fibres in the pericycle. Except at the apex of the one-year-old

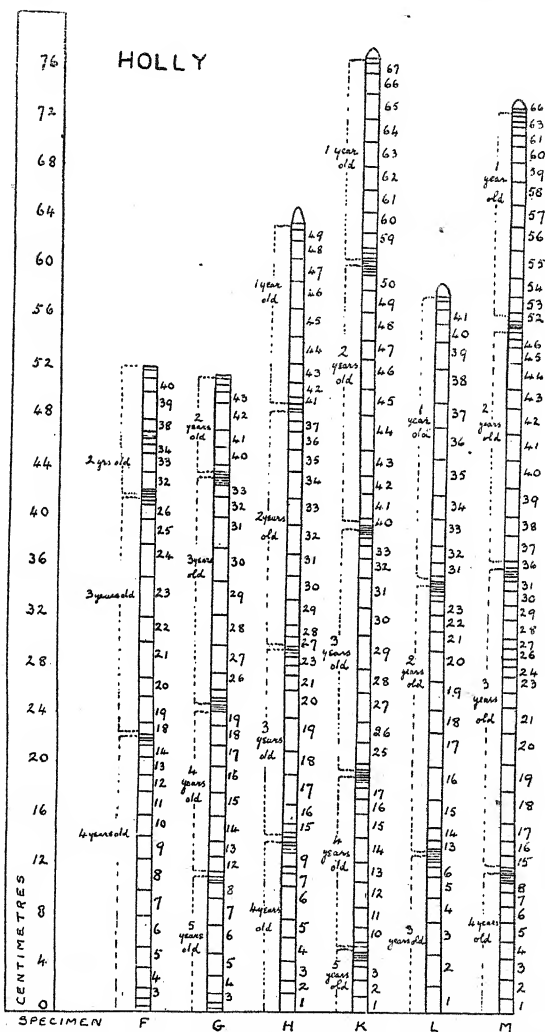


DIAGRAM II.

shoots, the protoxylem elements are inconspicuous; being obscured by disintegration products due to pressure. In general the spring wood of the first year contains numerous large water-conducting elements immediately outside the primary xylem, while the autumn wood consists largely of fibres; but just below the junction of each petiole, the first-year wood contains a well-defined radial group of very numerous small water-conducting elements,

which exert a modifying influence on the analysis of the wood in sectors—that is to say, a sector through one of these groups must be combined with sectors on different radii, in order to obtain a true average view of the wood-constituents in those particular sections. The first-year wood in each annual shoot forms a cone, and the constituents of the narrow apex differ considerably from those of the wide base (see Diagram III). The

HOLLY.

Longitudinal Section.

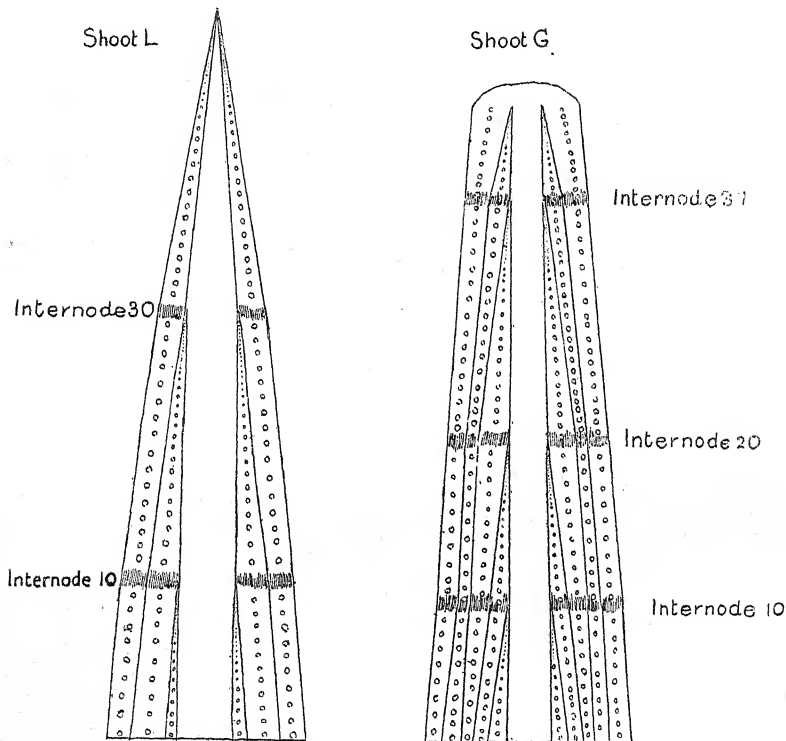


DIAGRAM III. To show comparative uniformity of outer rings of wood as compared with the first ring in each yearly segment.

second annual ring contains larger water-conducting elements than the first, those formed in the spring zone containing some of the widest individual elements to be seen. They are arranged more or less in radial rows or groups, with intervening radial patches of fibres. The third-, fourth-, and fifth-year woods are all very similar on a general inspection of transverse sections, showing wide water-conducting elements and patches of fibres. Owing to their similarity at different levels in the stem, they modify the general average analysis of old shoots to a considerable extent, and partially eliminate the variations noticed in the cone of the first-year wood. In July, when the wood was cut, cambial activity is still in progress and the

outermost zone of wood contains elements not altogether differentiated. In all the conducting elements bordered pits can be seen in transverse section wherever they are in contact with living cells or with other water-conducting elements.

The fibres, in transverse section, are much smaller than the water-conducting elements, and have thick walls staining deeply with safranin. They show slightly bordered pits, where they are in contact with living cells, but not where they abut on water-conducting elements. The living cells are largest in the multiseriate medullary rays: they have thick walls, protoplasmic contents, and a small quantity of starch.

In longitudinal section the water-conducting elements of the primary xylem are found to be long narrow vessels with spiral and annular thickenings, which do not separate into segments on macerating, but appear as long tubes without obvious remains of cross-walls. They are mostly under a centimetre in length. The conducting elements of the secondary xylem are elongated segments, about thirty to forty times as long as they are broad. The end-walls lie in an obliquely radial plane and are pierced by narrow, scalariform perforations, separated by numerous narrow bars. Thus they form continuous vessels, which separate into segments on macerating, but on injection appear as tubes which rarely reach  $3\frac{1}{2}$  cm. in length. No segments with unperforated cross-walls were observed, except in the junction tissue between the wood cylinder and the petiolar strands, where numerous irregular tracheides are to be found. The bordered pits are abundant wherever the vessels are in contact with living cells: they are frequently accompanied, especially in the outer zones of old stems, by spiral and reticulate thickenings.

*Method.* The general method employed for the analysis of the wood is that put forward by Miss Holmes<sup>1</sup> in her paper on hazel-wood. The internodes of each shoot were numbered from base to apex and transverse sections made in the midst of certain internodes. The sections were stained with haematoxylin and safranin and first examined under a very low power of the microscope (2-inch objective). The limits of the wood were traced on paper by means of a camera lucida at a magnification of 21 diameters. By means of a planimeter the true area of the wood was determined and calculated in square millimetres. Subsequently the wood was examined under a high power of the microscope (one-sixth inch objective), and again, by means of a camera lucida, an exact representation of the cavities of the water-conducting elements in a sector of known area was drawn on millimetre-squared paper. From this drawing the total area of all the cavities was obtained by counting the square millimetres which they contained. The number of elements in the known sector was also counted. Thus the

<sup>1</sup> M. G. Holmes: A Study in the Anatomy of Hazel-Wood with Reference to Conductivity of Water. *Ann. Bot.*, vol. xxxii, 1918.



proportion existing between the area of the cavities and the area of the known sector of wood was obtained. By dividing the total area of cavities by the square of the linear magnification and combining this with the true area of wood, the total area of the cavities in a complete transverse section was easily calculated. Further data which were calculated were the average area of the cavities and the number of cavities per square millimetre.

*Accuracy.* Owing to the comparative smallness of the field under a high power of the microscope, only a small sector of the wood can be examined at once. Neither in *Rhododendron ponticum* nor in holly is any such sector typical of a whole transverse section. The variability of the wood on different radii is due in *Rhododendron ponticum* to (i) the two-sidedness of the stem, (ii) the existence of tracts of living cells associated with the insertion of the petioles; in holly the variability is due to the living cells and small vascular elements associated with the insertion of the petioles. Theoretically, perfect accuracy can only be obtained by ascertaining the cross-area of every water-conducting element in every section; the laboriousness of this being evident, it was found that the approximation arrived at by combining three or five sectors gave satisfactory results.

*Results.* The results given by the above-described drawings and calculations are represented by a series of graphs. In all cases the internodes numbered from base to apex are plotted along the horizontal line, an equal interval (0.2 cm.) being taken for each internode, irrespective of its length. A special vertical scale is chosen for each set of figures according to the following plan:

A. Total wood area in sq. mm.	1 cm. represents 5 sq. mm.
B. Total cavity area in sq. mm.	1 cm. represents 1 sq. mm.
C. Percentage of wood occupied by cavities	1 cm. represents 2 per cent.
D. Average area of cavities in sq. $\mu$	1 cm. represents 40 sq. $\mu$ .
E. Number of cavities per sq. mm.	1 cm. represents 200.

By plotting points for each set of figures against the chosen internodes, a series of curves are obtained which show how they vary at different levels of the stem. The members of the series are indicated by the letters assigned to the plan of vertical scales put out above. The curves for the different shoots of *Rhododendron ponticum* are illustrated in Diagrams IV to XIII: the curves for the shoots of holly in Diagrams XIV to XVII.

Curve A (total area of wood in sq. mm.). In general this is a smooth curve descending from base to apex. The descent is due to the general tapering of the stem and the increase in the relative area of the pith in the higher levels. In *Rhododendron ponticum* the descent is generally more rapid towards the base than in the middle and apical regions, but this is not generally apparent in the holly. Of the rhododendron shoots B1 is the most massive, showing a wood-area of 32.2 sq. mm. at the base; in general the leading shoots are thicker than the lateral: in some cases

(C<sub>3</sub>, C<sub>1</sub>, A<sub>1</sub>) there is a flattening of the curve at the beginning of the leafy region of the stem, where the general tapering is influenced by the close succession of the petioles and the shortness of the internodes. The shoot A<sub>3</sub> has the lowest figure for wood-area (8.2 sq. mm. at the base). Among the holly twigs it should be noticed that in Shoot L the wood-area reaches 24.6 sq. mm. at the base of the three-year-old shoot. In Shoot H it is only 14.9 sq. mm. at the base of the four-year-old shoot.

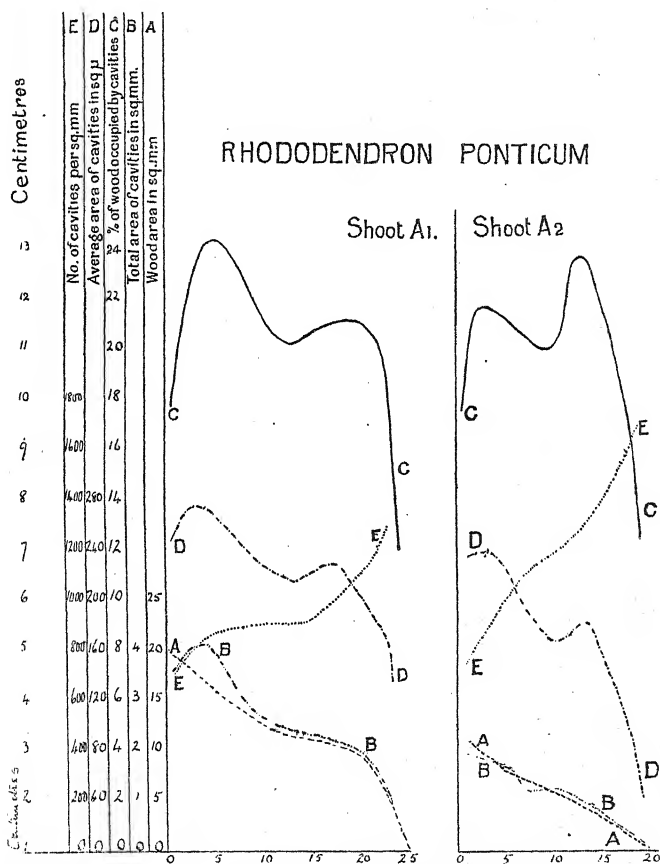


DIAGRAM IV. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.).

Curve B (total cavity area in sq. mm.). This curve gives (as far as is possible from a consideration of transverse sections) a measure of the absolute conductivity of the wood—i.e. of the volume of water which it can transmit in any known period of time. Its direction is associated with that of Curve A, consequently there is a general descent from base to apex. There are peculiarities in the curves for the rhododendron shoots, different from those seen in holly. In the shoots of *Rhododendron ponticum*, in

which the mass of supporting fibres is limited to the extreme base, and in which there is a sudden increase of vessels in the first elongated internodes, the curve tends to rise slightly at first, because the increase in the total cross-area of the vessels more than makes up for the slight decrease in area of the wood. This preliminary rise is most marked in Shoots E4, E2, D1, and A1. Similarly, at the beginning of the foliar region, where there is a slight increase in the size of the vessels, we may have either

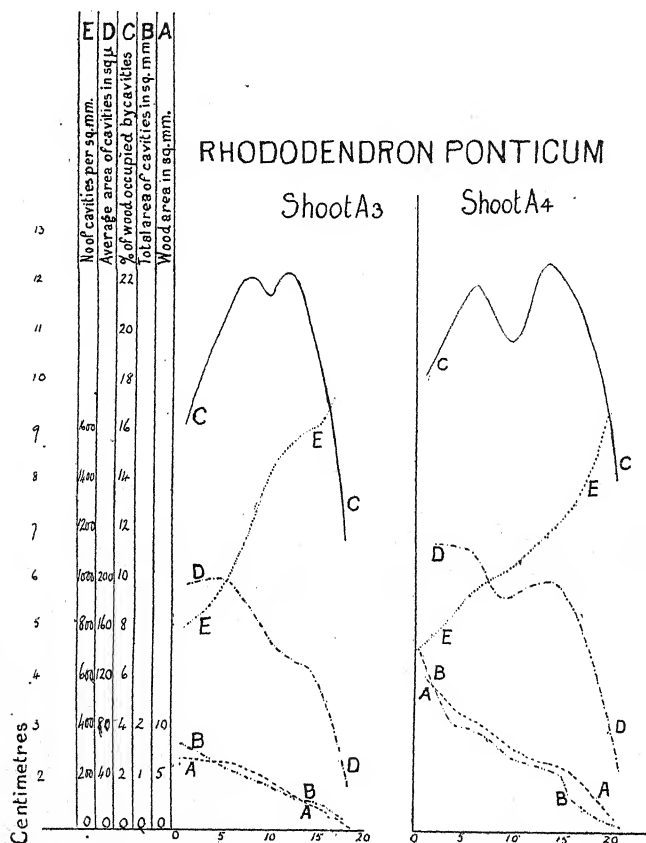


DIAGRAM V. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

a flattening or slight ascent of the curve, because here the tapering of the stem is slight and any increase in the actual area of the vessels becomes noticeable in the total area in the section. The range of actual figures is from 5.9 sq. mm. in B1 to 1.4 sq. mm. in A3. In the shoots of holly the general descent of the curve throughout the length of the twig is interrupted by more sudden descents at the yearly joints, which begin at the apex of one year and extend into the lowest internodes of the next. These sudden descents are due to a decrease in number of water-conducting

elements at these points (see Curve E). The slight variations in the form of the curve in different shoots and different years are due to the varying number of internodes, to the rate of decrease in the wood-area, and to the changes in size and number of the water-conducting elements. In Shoot F the curve is nearly flat at the beginnings of the yearly segments and descends at their apices: it terminates nearly flat owing to the abortion of the apex. In Shoot G the curve is very similar, but there is a slight

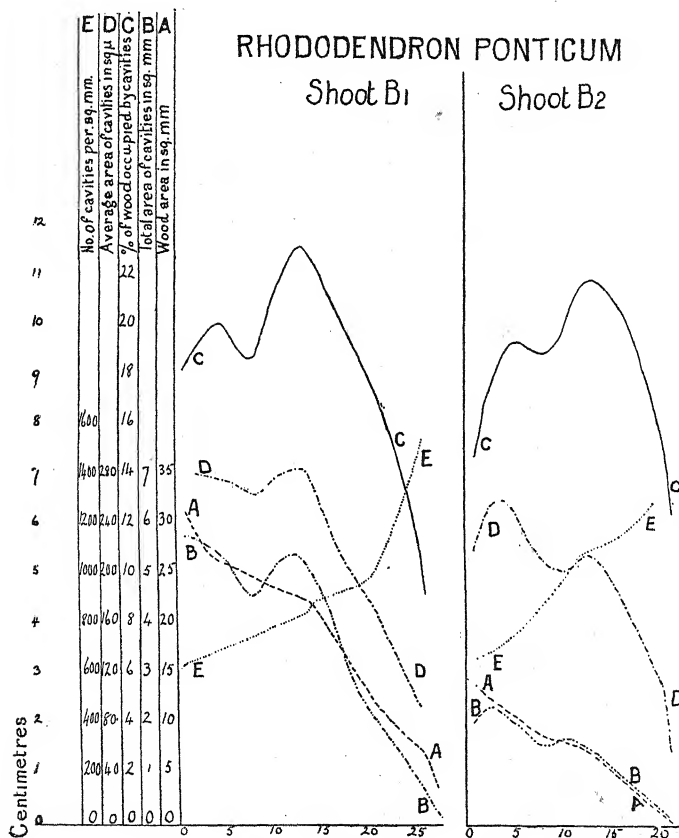


DIAGRAM VI. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

departure from the general descent in the middle of the three-year-old segment, due to the large number of water-conducting elements at that region. In Shoot H the general descent is very gradual, because the stem tapers very gently and there is no great variation in the cross-area of the water-conducting elements throughout the very long stem, but the yearly segments are well marked. In Shoot K the yearly segments are again well marked, and, though they are similar in form, the actual figures vary considerably. In the four-year-old segment the wood-area is decreasing

slightly, but the more numerous water-conducting elements in its middle region causes the curve to be slightly rounded: this is even more marked in the three-year-old segment, where the wood-area decrease is slight and the water-conducting elements are very numerous in the middle region. In the two-year-old segment the wood-area decrease is again steeper, but

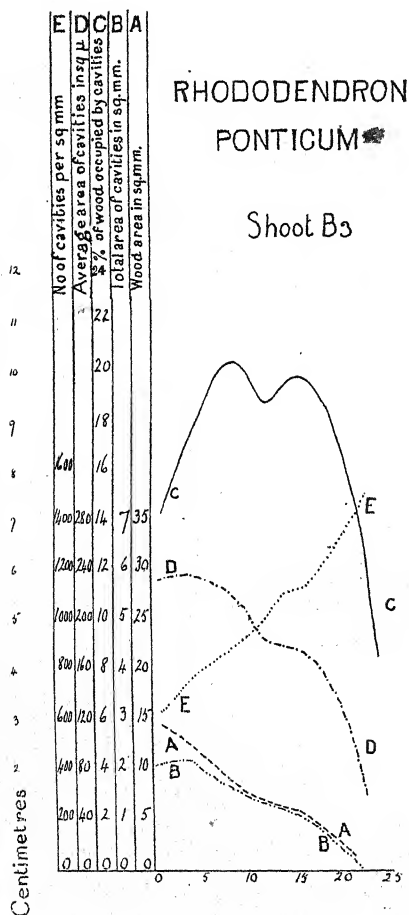


DIAGRAM VII. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

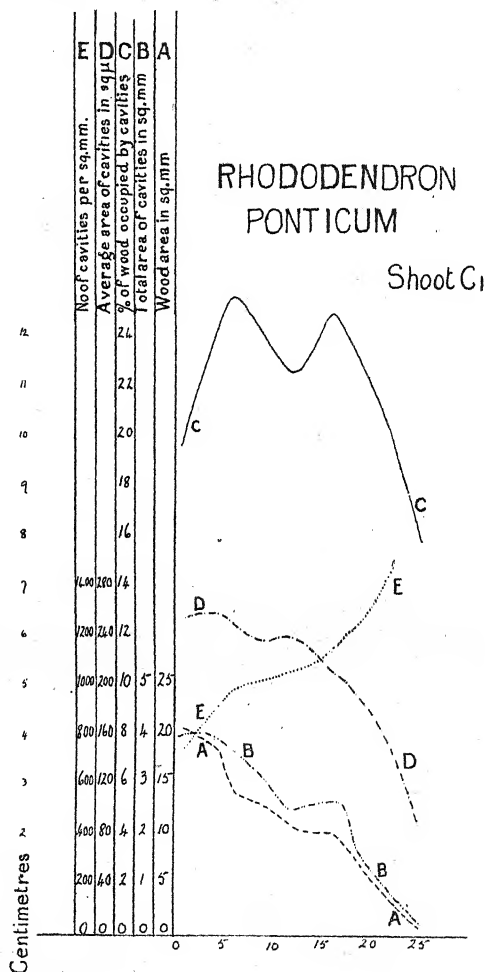


DIAGRAM VIII. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

the change in the number of conducting elements still causes the curve to be rounded. In the one-year-old segment there is only a general decrease and no rounding of the curve, consequent on the decreasing size of the elements and the decrease in wood-area. In Shoot L, which was a rapidly growing, well-favoured specimen, there is a marked fall of the curve at the end of the three-year-old segment, but the rest of its descent is gradual.

Curve C (percentage of the wood occupied by the cavities of water-conducting elements). This curve illustrates, as far as is possible from the consideration of transverse sections, the anatomical basis of the variations in the water-conducting powers of the wood, and thus is to be correlated with the specific conductivity—i.e. with the volume of water transmitted

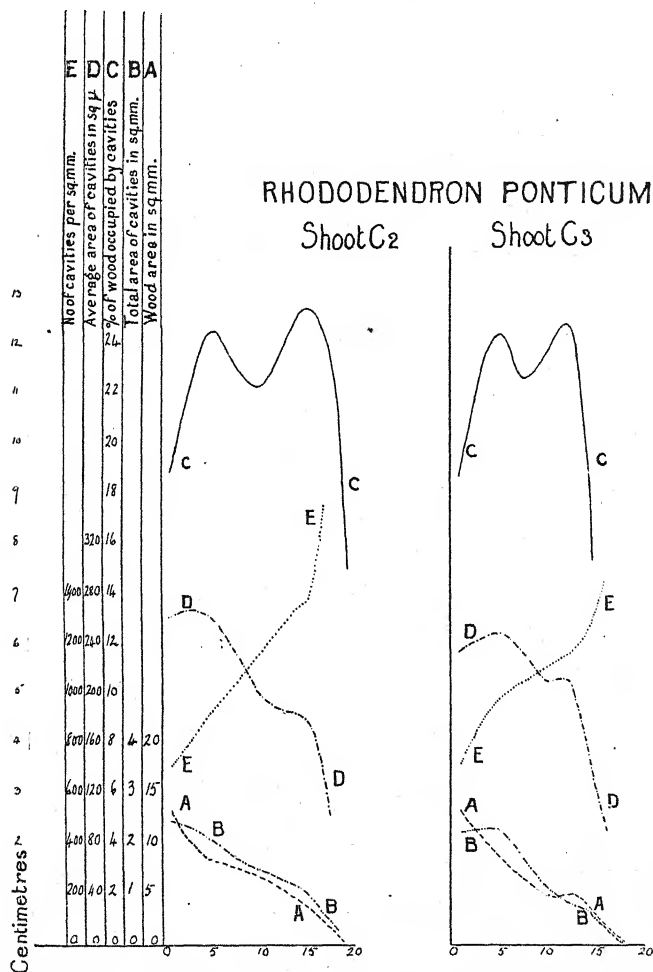


DIAGRAM IX. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

by a length of 15 cm. per 1 sq. mm. of wood as seen in transverse section. There are considerable differences in the curves exhibited by the one-year-old shoots of *Rhododendron ponticum* and the three- or four-year-old shoots of holly, but in general the curves rise from the base and fall away again to the apex. In rhododendron the curves may be described and explained as follows: The steep rise from the base through the lower internodes

is succeeded by a descent below the leafy nodes, but this gives place to another ascent before the rapid fall to the apex. The first steep rise indicates a great increase in the number of vessels in the first elongated internodes (see Curve E): these supplant the fibres which act as supporting tissue, at the extreme base. They form the vascular tissue which supplies the young transitional leaves which unfold rapidly in the first warm spring weather. The subsequent descent in the curve is due to the decrease in average size of the vessels (see Curve D), and also in some cases to a slight falling off in the general increase in number. The second rise in the curve is due sometimes to a slight increase in the size of the vessels at the beginning of the foliar region and to the general increase in number, but as the large vessels are mostly at the inner margin of the wood and are laid down before the leaves expand, this cannot be correlated with the activities of the leaves. The final fall of the curve is due to the rapid decrease in the size of the vessels as the apex of the stem is reached. The irregularities of the curves in individual shoots are seen to be considerable, but they appear to be chance variations in the differentiation of permanent tissue, rather than a response to the mechanical and transpiring necessities of the leaves. These irregularities make it evident that a considerable latitude in specific conductivity is to be expected, and this agrees with the experimental results obtained by Professor Farmer, which are detailed below. The levels of the curves are very similar in the shoots of Group A and Group B, and this agrees with the similarity of the figures for specific conductivity. These latter lie between 11.2 and 13.9, while the means of the percentage figures in the segments of the shoots selected for experimental purposes lie between 20 and 22. This correlation can be traced throughout by reference to the following table:

<i>Shoot.</i>	<i>Experimental Sp. Conductivity.</i>	<i>Mean Figures for the percentage of Wood occupied by Cavities in the Experimental Segments.</i>
C 1	16.4	24.0
C 2	15.0	23.6
C 3	16.9	24.0
D 1	18.6	25.8
D 2	18.2	25.5
D 3	20.8	26.5
E 2	21.8	27.9
E 3	18.6	25.8
E 4	20.7	27.2
E 5	19.4	26.0
E 6	15.5	23.6

In the holly shoots the C curves show a general rise from the base to a maximum somewhere near the apex of either the two-year-old or one-year-old segments, and then fall away to the terminal bud, but, as in Curve B, the general trend is obscured by sudden interruptions at the yearly joints. The position of the maximum indicates that the first- and second-year wood



is richer in vessels than the outer rings. If a mean line is drawn touching the maxima of successive yearly segments, it will be found to have a general inclination upwards to the middle of the two-year-old or one-year old segment. The whole range of the line lies between 7.5 and 10.5, and the inclination becomes less and less in the older segments. This agrees with Professor Farmer's experimental results for the specific conductivity, which he found to vary between very small limits ( $9 \pm 2$ ) without any divergences due to age. Following the curve in greater detail, it will be seen that in each year there is a rise from the base to the middle and a fall to the apex. At each yearly joint there are several internodes (between the winter-bud scales) where the percentage figure is relatively low. In discussing Curve E, it will be found that this is due to a great decrease in the number of water-conducting elements at these regions, their place being taken by fibres. It is a matter of common observation that twigs are hardest to break or cut at the yearly joints. In Shoot F the figure for the percentage at the base of the four-year-old segment is 7.1 and the highest is 9.5, near the apex of the two-year-old. The descent at the upper end is only to 5 per cent. because there is no one-year-old shoot developed. In Shoot G the percentage is 6.9 at the base of the five-year-old segment and 7.9 in the middle of the three-year-old. The range here is seen to be slight, indicating that the percentage figure is not materially affected by age, once maturity is established. In Shoot H the yearly phases are very clearly marked and also the closeness between the maxima and minima in different years. The total range is from 6.0 at the base of the three-year-old segment to 10.0 in the middle of the two-year-old. Shoot K, the longest and stoutest of the set, is the richest in conducting tissue: even at the yearly joints, the percentage does not fall below 6.0 and it rises to 11.3 in the middle of the three-year-old segment. This is due to large numbers of vessels and not

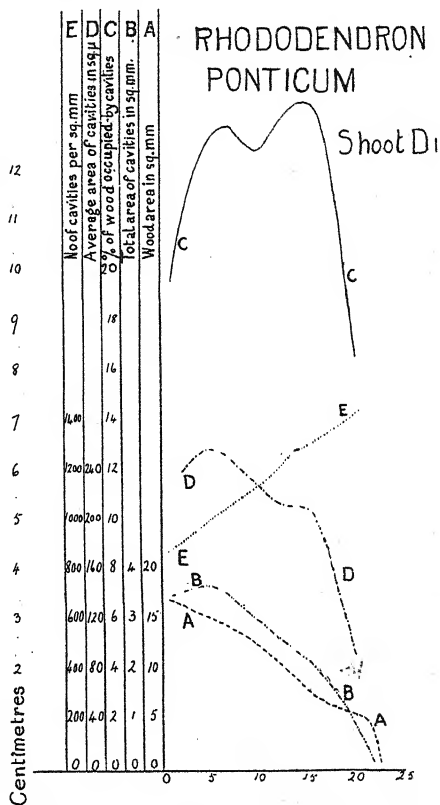


DIAGRAM X. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

to variations in their cross-area. In Shoot L, which has very long internodes and appears rapidly grown, the range between the maxima is very slight—7.9, 8.1, and 8.5 being the figures found in successive yearly segments: the readings at the joints are 6.8 and 7.4 and the rich conducting-tissue is maintained until the last two internodes. In Shoot M the figures are normal in the lower yearly segments, but the one-year-old shoot was very

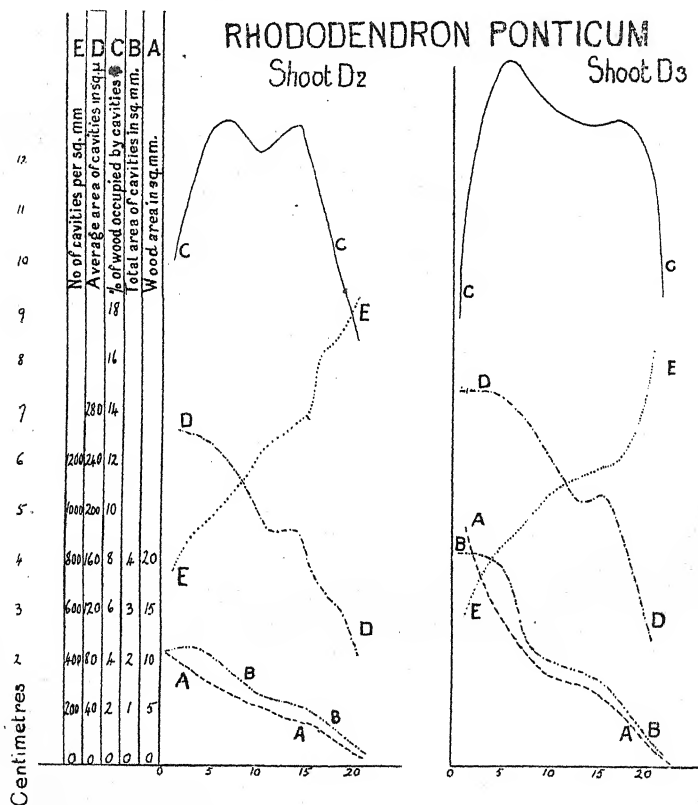


DIAGRAM XI. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

soft and there is not the usual displacement of vessels by fibres in the joint between the one-year-old and two-year-old segments.

Curve D (average area of cavities in sq. μ). In both *rhododendron* and *holly* the curve descends from base to apex, indicating that the average cross-area of the vessels is largest at the base of the shoots. In the lower internodes, the new vessels which replace the basal fibres are small in size and their number helps to decrease the average area, though there are still numerous large vessels present. In the leafy region of *Rhododendron ponticum* a great number of larger vessels are found close outside the primary xylem, and this causes an increase in the average size, which

produces a change in the direction of the curve. This is the most marked in the B group. It is partly this increase in the area of the cavities which in some shoots causes the second ascent of Curve C; but it does not bring

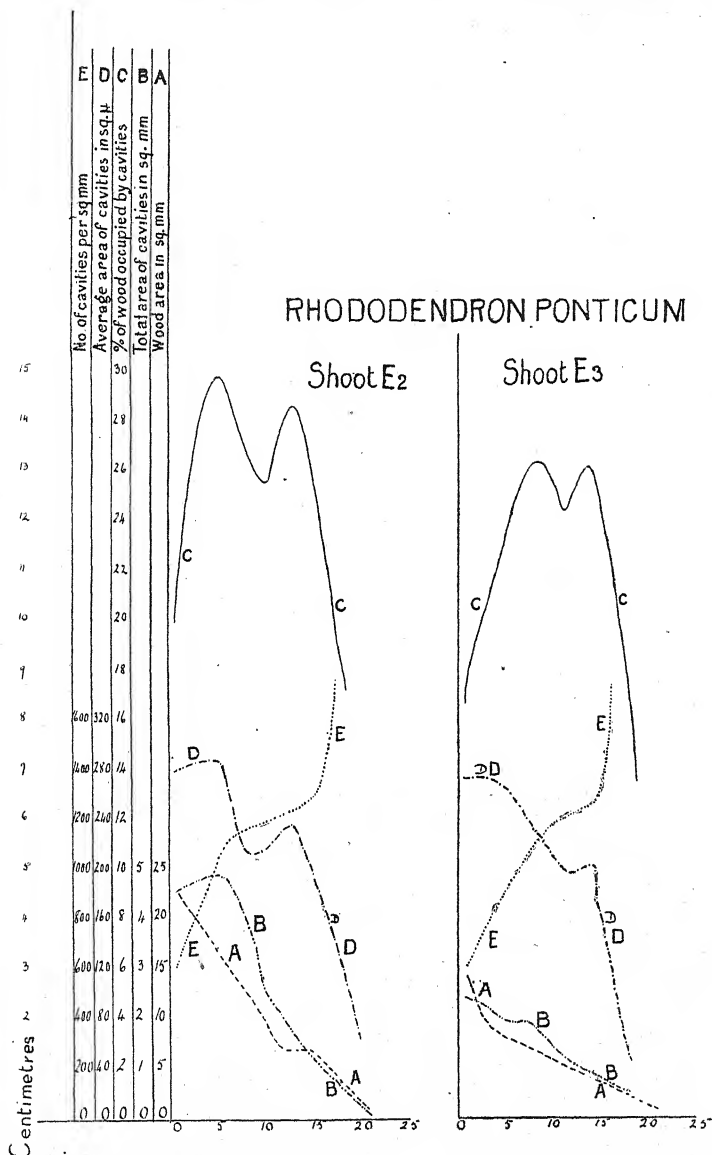


DIAGRAM XII. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

up the average to the figure reached at the base, because, owing to the decrease in wood-area, the small autumn vessels close to the periphery have a more marked effect on the general average. It should be noticed here

that the friction in vessels of small lumina is greater than that in large, and that this will affect the conductivity in the sense that, area for area, it will be lower in vessels which are narrow and numerous than it will be in those which are fewer and wider.

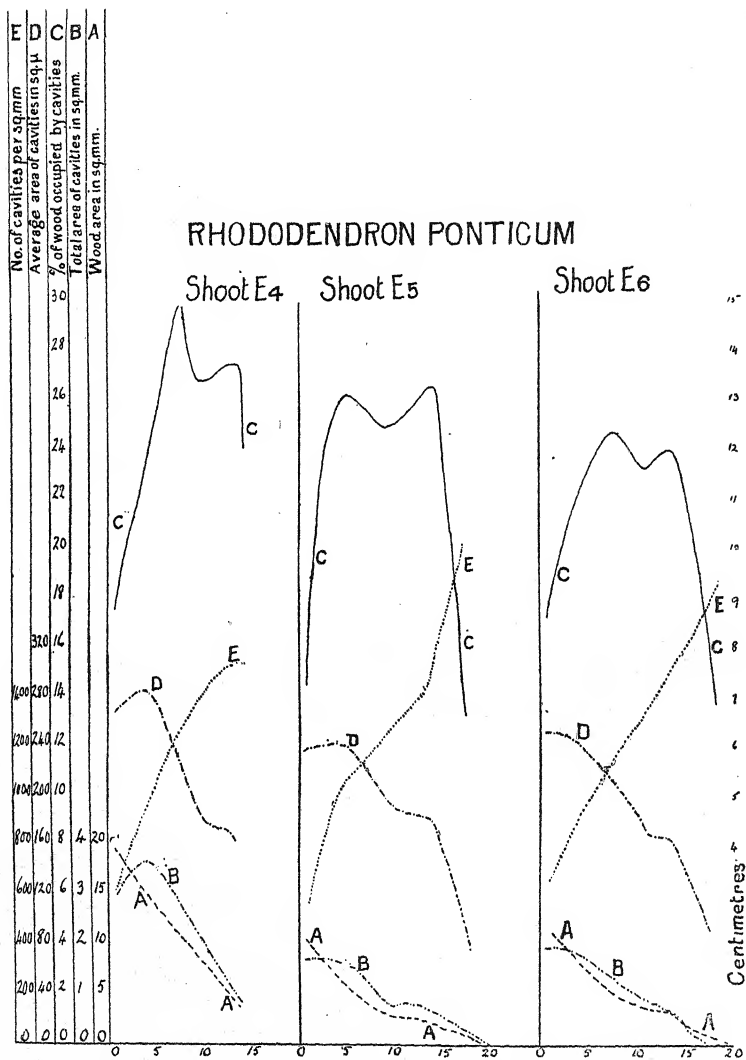


DIAGRAM XIII. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

In the holly the D curve is noticeable because it is hardly affected by the yearly joints as are the curves B, C, and E. The highest figures are found at the bases of the oldest shoots, and they become lower in the passage through successive years to the apex. This is due to the fact that the vessels in the outer rings of wood are, on an average, slightly

larger than those in the inner rings, but it is also true that in each annual ring the vessels in the more basal internodes of a year's growth are slightly

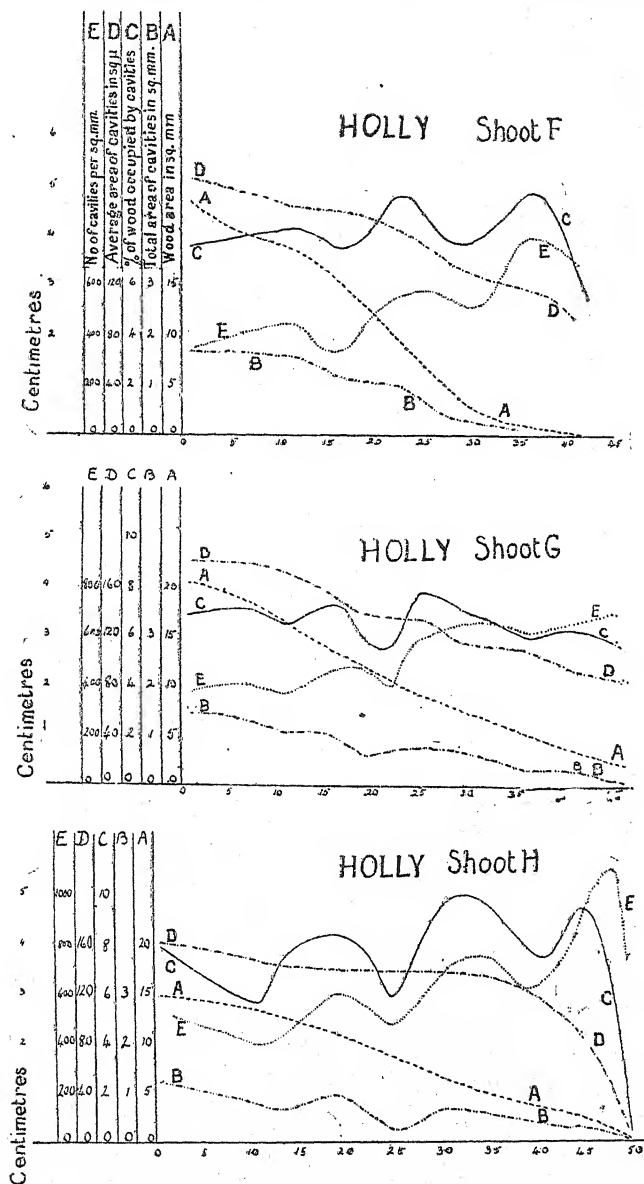


DIAGRAM XIV. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

larger than they are higher up. The last statement is especially true in the first annual ring, where the divergence is quite remarkable. This is illustrated in Diagram III. It is to be observed in the first annual ring

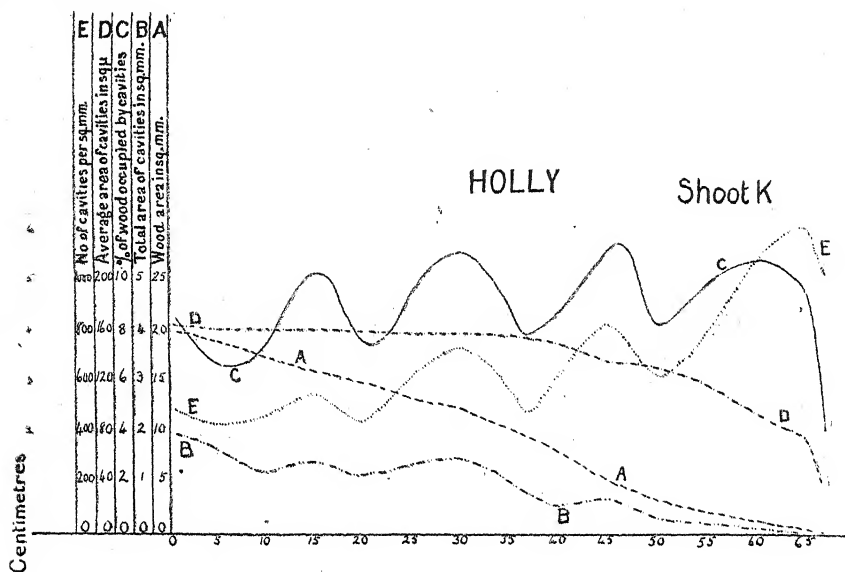


DIAGRAM XV. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

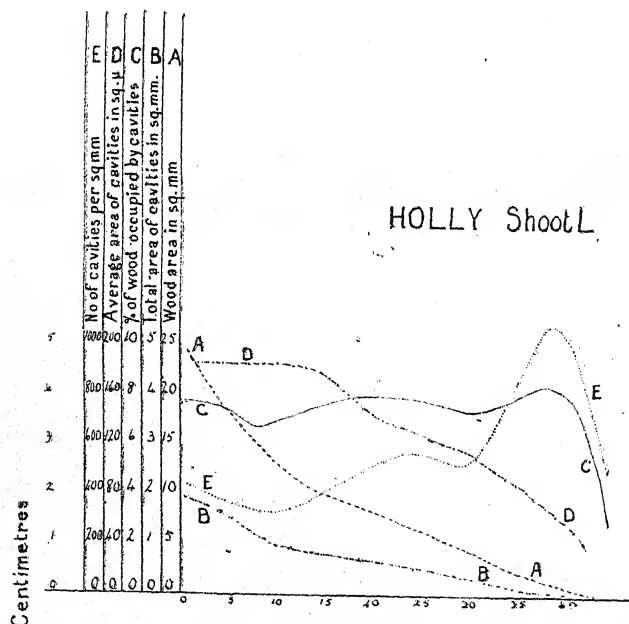


DIAGRAM XVI. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

that there are one or more large groups of very numerous small vessels occupying certain sectors. These are the conducting elements which connect with the strands of the petiole and the axillary bud: they exert

a considerable influence on the general average of any particular transverse section. The highest figures are found at the base of the four-year-old segment of Shoot F, where they reach 200 sq.  $\mu$ . For its age, however, the three-year-old shoot L has very large vessels, reaching an average area of 185 sq.  $\mu$  in the middle of the three-year-old segment, which is a much higher figure than is found in the corresponding part of any other shoot. In Shoot F the curve shows the general descent slightly flattened at the joints and terminating rather high up owing to the truncated apex. In Shoots H and K the curve falls gradually in the older segments, but becomes steep towards the apex. In Shoot L the curve descends more

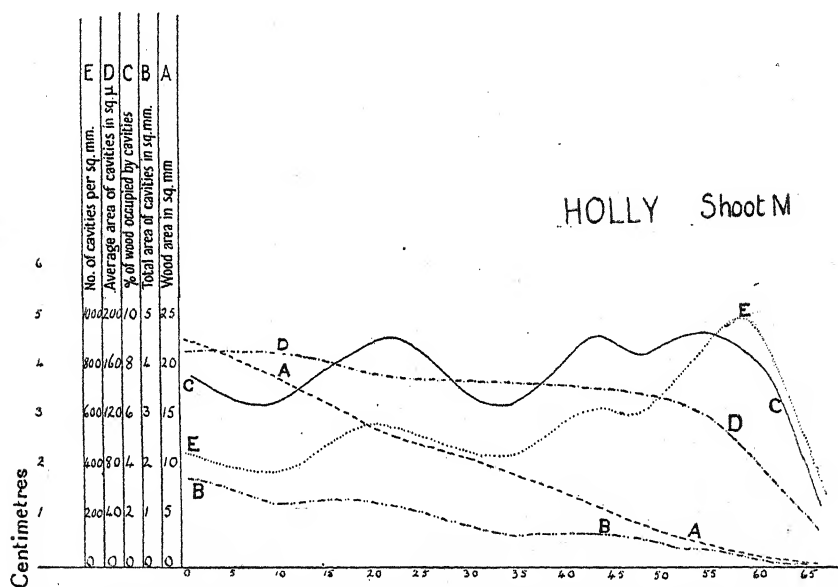


DIAGRAM XVII. Internodes numbered from base to apex. (Scale, 5 internodes = 1 cm.)

steeply, this being a young, rapidly grown specimen, where in the first annual ring of each year's segment there is a marked difference between the higher and lower vessels. Hence, there being no modifying influence of the more similar averages of the outer rings, we see the rapid descent of the curve, which is characteristic of a one-year-old shoot.

Curve E (number of cavities per sq. mm.). Speaking generally the curve shows a general rise from the base to a point near the apex. In *Rhododendron ponticum* the rise is steep at the base, flattens out slightly in the middle, and becomes steep in the apical internodes. The flattening in the middle indicates a slight falling off in the general increase in number and corresponds with an ascent in Curve D, showing that in this region there are smaller numbers of slightly larger vessels as compared with the region immediately



below. The rise at the base corresponds with the rise in Curve C, showing that the first increase in vascular tissue is due to an increase in number. The steep rise at the apex is not shared by Curve C, showing that here the increased number of vessels will not make up for their small lumina from the point of view of water-conduction. The figures calculated vary from 556 at the base of Shoot E 5 to 1,908 at the apex.

In holly the most marked characteristic of the E curves is the sudden fall at each yearly joint. The fall is due, as already indicated, to the large number of fibres developed in those internodes between the winter-bud scales. In reality, though they occupy several nodes, their influence in the length of the stem is very slight, because at these regions the nodes are much crowded together. The figures calculated vary from 330 to 755 in Shoot F and from 439 to 1,228 in Shoot K, so that we have among all the shoots a minimum of 330 and a maximum of 1,228 vessels per sq. mm. The individual variations in the form of the curves are not sufficient to warrant a separate description.

*Comparison of Results.* The general points of resemblance between the corresponding curves which have been figured for *Rhododendron ponticum* and holly have already been noted. They are also shared by the curves which have been figured elsewhere for the stool shoots of hazel and ash. It must be remembered, however, that whereas it is only the one-year-old wood which has been analysed for rhododendron, ash, and hazel, the observations extend through shoots of four or five years for holly. Thus, in comparing the curves, only the apical segments in Shoots H, K, L, and M of holly should be referred to.

The irregularities of the wood area curves (A) (most marked in rhododendron) have no great significance and do not warrant further description.

The C curves show considerable divergence in the four woods examined. In rhododendron and holly they rise steeply from the base of the one-year-old segments. The maximum in holly is reached more or less in the middle, but generally nearer the apex than the base. The maximum in rhododendron may be reached either nearer the base or nearer the apex: in several cases the maximum is reached nearer the apex on account of the increase in size of the vessels in the leafy part of the stem. The holly is a comparatively small-leaved plant, and the vessels in the stem which link on to those in the petiole are much smaller than the normal found at other levels, while in rhododendron this is not so. Also in the holly the separation of each year's growth into a leafless and leafy region is not nearly so marked as it is in rhododendron; the transitional leaves are comparatively few in number, and permanent foliage leaves may be found at the lower nodes; thus the anatomy in holly shows less variations in analysis than does the rhododendron.

The actual figures obtained for the C curve are higher for rhododendron than for holly, ash, or hazel: thus we have

Rhododendron	.	.	.	.	14.5 to 29.9 %
Holly	.	.	.	.	5.0 „ 10.6 %
Hazel	.	.	.	.	3.2 „ 20.2 %
Ash	.	.	.	.	1.6 „ 9.5 %

Professor Farmer's figures for the specific conductivity as determined experimentally are as follows:

Rhododendron (A 1 to E 6)	.	.	.	.	11.9 „ 21.8 %
Holly	.	.	.	.	7.0 „ 11.0 %
Hazel	.	.	.	.	22.0 „ 40.0 %
Ash	.	.	.	.	4.0 „ 24.0 %

There seems a certain difficulty in correlating these two sets of figures. The relation between holly and rhododendron is clear enough, and also that between hazel and ash, but when we come to compare the evergreens with the deciduous trees there is no similar relation holding. In rhododendron there is a higher percentage of the wood occupied by water-conducting elements than there is in the hazel, but the hazel shows the higher figures for water-conduction as carried out experimentally. The explanation of the anomaly may partially be found in the lengths of the continuous vessels, in the lengths of the vessel-segments, and in the type of perforation which renders the segments continuous. The very considerable variation in the lengths of the vessels (5-16 cm.) in rhododendron is to be correlated with the great variation in specific conductivity: short vessels with numerous unperforated cross-walls increase the resistance to the passage of water: similarly, short segments and small scalariform pores increase resistance and decrease conductivity. Similar considerations hold good for the conducting tissue of the holly.

In comparing the relative average cross-areas of the vessels in the four types of wood examined, we have the following data:

Rhododendron	.	.	.	298 sq. $\mu$ -98 sq. $\mu$	} Calculated from figures for average diameters.
Holly	.	.	.	200 „ -52 „	
Hazel	.	.	.	422 „ -18 „	
Ash	.	.	.	607 „ -7 „	

Thus the vessels in the stool shoots of the deciduous ash and hazel have on the whole a much larger average cross-area than those of the evergreen rhododendron and holly: this accounts in a small degree for the high conductivity of the hazel and its relatively low percentage figures, because friction and resistance are less in wide vessels than in those with small lumina. Similarly, the high percentage figures in the rhododendron are partially explained by the smaller lumina of the individual vessels.

In comparing the values for the number of water-conducting elements per sq. mm. we have the following :

Rhododendron	.	.	.	556-1908
Holly	.	.	.	331-1195
Hazel	.	.	.	115-4000
Ash	.	.	.	32-633

From this little can be deduced except that the extreme figures for hazel cannot be representative of any great length of the stem, because, although the average cross-area of its water-conducting elements reaches a higher maximum than in rhododendron, and although the figure for the maximum number of conducting elements is extremely high, yet the values for the C curve fall lower than they do for rhododendron. Obviously the balance between size and number for securing maximum conductivity is very delicate and is achieved in hazel rather than in rhododendron, but in the end we are forced to conclude that it is the length of the vessels rather than this balance which is the important factor in conductivity.

#### CONCLUSION.

The results obtained from the quantitative analysis of the wood in *Rhododendron ponticum* and the holly have been described and correlated with the results given by experiments on their specific conductivity. An attempt has also been made to compare the ascertained data with those given elsewhere for the stool shoots of hazel and ash. The comparison indicates that the evergreen shrubs examined have, as a characteristic of their wood, vessels of a smaller bore and shorter length than those of the deciduous hazel and ash. At the same time all four agree in the fact that there is a general decrease in absolute conductivity from the base upwards in each year's growth, while there is an increase in specific conductivity from the base up to a point near the apex of each year's growth.

In conclusion I wish to express my thanks to Professor Farmer both for the suggestion of this research and for the assistance which he has rendered.

# Besleria lutea, Linn., a New Example of Water-calyx.

BY

MONTAGU DRUMMOND, B.A., F.L.S.,

*Lecturer in Plant Physiology, University of Glasgow.*

*BESLERIA lutea*, Linn., is a small Gesneraceous shrub, which grows in moist mountain woods in Jamaica and several other West Indian islands, as well as in north-eastern South America. It occurs plentifully in the vicinity of Cinchona, in the Blue Mountains of Jamaica, where the peculiar swollen appearance of its calyx attracted my attention during my stay at the Hill Garden in the late summer of 1913. A closer examination of flower-buds showed that the tube of the gamosepalous calyx is greatly inflated, especially towards its base, so that the corolla and essential organs—which lag far behind the calyx in development—are, at this stage, enclosed in a bell-shaped cavity of considerable size. In the bud, this cavity is completely filled with a clear watery liquid, which is prevented from escaping, chiefly by the circumstance that the closely apposed, connivent lobes of the calyx-limb are slightly twisted around one another. The margins of the calyx-lobes further interlock by means of cellular sutures.

The calyx-cavity remains filled with 'water' after the corolla has burst through the tip of the calyx, and even after the flower is fully open. The application of gentle pressure to the calyx of a flower in full bloom causes liquid to exude from its apex. During the ripening of the fruit the calyx becomes still more distended, and assumes a globose shape, thus keeping pace with the enlargement of the ovary to form a spherical berry. Some considerable time before the fruit matures, liquid disappears entirely from the calyx-cavity, which thenceforth contains air.

The calyx is a somewhat massive structure, but its anatomical construction is simple. The outer epidermis is small-celled, and bears scattered simple hairs composed of from one to three thick-walled, cylindrical cells, the terminal one of which tapers to a point. A few stomata are present on the outside of the young calyx, but these are very soon replaced by numerous small lenticel-like structures. The peripheral parts of the mesophyll consist of closely-packed, slightly collenchymatous elements; the central portion is thin-walled and well provided with intercellular spaces. The inner epidermis is large-celled, quite devoid of stomata, and bears numerous large capitate

glands all over its surface, but especially in the vicinity of the vascular bundles, which lie at the adaxial edge of the central mesophyll. These glands agree in general structure with the trichome-hydathodes of *Clerodendron Minahassae*, Teijsm. and Binn., as described by Koorders (Ann. Jard. Buit., xiv, 1897), but offer some features of interest which will be dealt with in a subsequent communication.

There is every reason to suppose that the liquid contained in the calyx-tube is secreted by the capitate glands of the inner calyx-epidermis. In all the cases of water-calyx previously described, glandular trichomes occur in a similar position, although in certain of the instances investigated by Koorders (l. c.), e. g. *Parmentiera cereifera*, Seem., and *Crescentia Cujete*, Linn., the outer epidermis of the corolla is also provided with similar structures. The corolla of *Besleria lutea*, Linn., possesses no features suggestive of a special water-secreting capacity, and the relatively feeble development of its vascular system tells against any such activity on its part. The calyx, on the other hand, has a well-developed system of vascular strands, the glandular hairs are most plentiful in the neighbourhood of these strands, and the intervening parenchyma tends to arrange itself in rows radiating from the bundles towards the glanduliferous strips of the inner epidermis.

The physiological or ecological value of water-calyces is obscure. Koorders (l. c.) regards water-calyx as a device for protecting the other parts of the flower against desiccation. That may quite probably be the function, or one of the functions, of the calyx in such a tree as *Spathodea campanulata*, Beauv., which, as Treub (Ann. Jard. Buit., viii, 1890) has remarked, bears the large flowers massed together at the ends of its branches, where they are regularly exposed to intense insolation. This explanation is, however, not so readily applicable to *Besleria lutea*, Linn., which, in Jamaica, inhabits a very humid and only moderately hot region, and which, moreover, is generally to be met with in the shade of taller shrubs or trees. I found it, for example, growing luxuriantly on the banks of the Mabess River, in a deep, densely wooded valley, where the atmospheric humidity is so constantly high that epiphyllous growth is extraordinarily prevalent. Lagerheim (Ber. d. deutsch. bot. Ges., ix, 1891) has suggested that water-calyx may serve as a protection against perforation of the corolla by humming-birds and other nectar thieves. At Cinchona, open flowers of *Besleria lutea*, Linn., are very frequently injured by some animal, which lacerates the limb of the corolla; but I did not observe the plant being visited either by humming-birds (two species of which are very common in the neighbourhood) or by any other animal.

I had no opportunity of determining the chemical composition of the liquid secreted by the calyx, but, according to my field-notes, it is clear and slightly mucilaginous, when fresh, and has a distinctly saline taste.

So far as I am aware, the present note is the first record of the occurrence of a water-calyx in the Natural Order Gesneraceae. The closely allied Bignoniaceae, however, include most of the plants with water-calyces so far described. The genus *Besleria* comprises some sixty species, spread over the West Indies and Central and South America, more than a quarter of the whole being natives of Brazil. Few of these are to be met with in cultivation, as their flowers are generally small and dully coloured, and the habit of the plants is ungraceful. *B. Imray*, Hook., is figured in 'Bot. Mag.', Tab. 6341, and its calyx, as depicted there, strongly recalls that of *B. lutea*, Linn. A comparative study of the calyx within this large genus would in all probability throw fresh light on the origin and significance of water-calyces in general.

I wish to record my indebtedness to the Royal Society of London for a grant in aid of the expedition on which the material of *Besleria lutea*, Linn., was obtained.





## NOTE.

**A SIMPLE ROOT AUXANOMETER.**—None of the standard methods for measuring the rate of growth of roots is convenient for use with large practical classes in that they involve either the use of a horizontal microscope or other expensive apparatus, or else the magnification of the movement is not sufficient to allow the rate of growth to be determined in the course of a few minutes.

A simple method has been used in this laboratory and has proved entirely satisfactory for class purposes. It is sufficiently simple and straightforward for use by elementary students, while, with certain modifications, it can be made to yield results of such accuracy as to warrant its employment for more serious purposes.

In its simplest form the apparatus required consists of a gas jar, A, fitted with a cork, B. Through a hole in the cork a glass rod, C, runs freely. A long pin passes through the cork and impales a seedling, D, with a straight root.

The method of procedure is as follows. The glass rod having been inserted through the hole in the cork, as shown in the sketch, a convenient quantity of water is poured into the jar and the height of the seedling adjusted by means of the pin until the root tip just touches the surface of the water. Upon lifting up the glass rod a short way, the level of the water in the jar falls and leaves the root tip above the surface. The rod is then slowly lowered until the root tip just touches the water surface, and the position of the rod in relation to the top of the cork through which it passes (or to a wire pointer) is recorded by making a mark upon it. The apparatus is left for 5 or 10 minutes and a fresh reading taken: this will be found to differ from the previous one by 2 or 3 cm. or more of the glass rod in the case of an actively growing root.

The exact moment at which the root tip touches the water surface is very evident, owing to the sudden rise of the water round the root by surface tension—it is much more evident than the moment of contact of a metal pointer with a surface of mercury which does not 'wet' it. If the glass rod is lowered slowly and carefully, readings of its position should vary less than a millimetre.

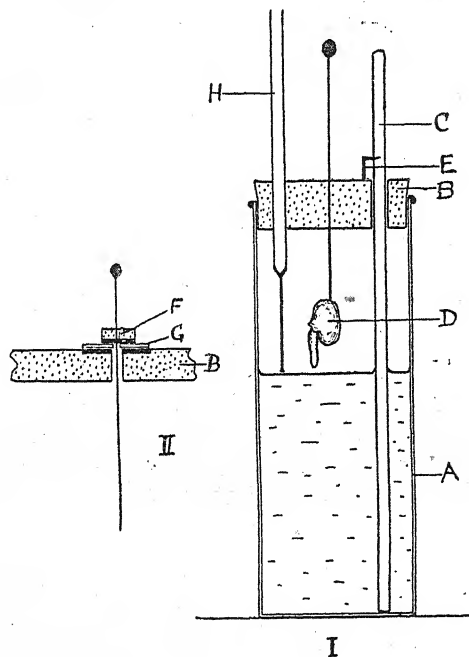
The magnification involved can be determined by measuring the inside area of the cross-section of the jar ( $S$ ) and the area of the cross-section of the glass rod ( $s$ ): the actual elongation of the root will be the distance apart of the marks on the glass rod  $\times \frac{s}{S}$ . For example, with a glass rod 5 mm. in diameter and a jar 5 cm. in diameter, the magnification will be 100 times, and every millimetre the glass rod is lowered will cause the water level to rise 0.01 mm. in the jar. It is a convenience to employ a rod graduated in millimetres: the absolute amount of growth of the root, if required, is then obtained by dividing the reading on the glass rod by the magnification coefficient.

The sensitiiveness of the apparatus can be varied within wide limits by varying the diameters of the jar and rod—the magnification increasing as the square of the radius of the jar and inversely as the square of the radius of the rod.

Care should be taken that the root tip is kept away from the sides of the jar and from the near neighbourhood of the glass rod, as the water surface will be considerably curved there. It is also desirable to have a cylinder of cardboard or black paper to slip over the jar to shield the root from the light.

The inaccuracy due to water adhering to the glass rod when it is raised was found to be negligible.

The following elaborations are suggested where greater accuracy is required or when prolonged observations are contemplated.



(1) The difficulty of accurately measuring the cross-section of the jar at the level of the water surface makes it impossible to obtain really precise absolute readings by the method of calculation suggested above. The following method of calibration is suggested:

An additional small hole is bored through the cork, through which is inserted a long pin (see Fig. II). A small disc of cork, *F*, too large to pass through the hole, slides on the pin, so that the distance of the pin-point from the water surface can be adjusted. The position of the glass rod which just causes contact of the water surface with the pin-point is determined. A thin washer, *G*, of known thickness is then inserted under the supporting cork disc on the pin, and a fresh determination made. From these data the actual rise in water level caused by immersion of a given length of the rod can be calculated.

It would no doubt be advantageous for the under surface of the cork disc and the upper surface of the cork on which it rests to be faced with some harder and more accurately plane surface than cork. A microscope cover-glass with a hole drilled through the centre should serve. Another drilled cover-glass forms a convenient washer.

(2) If the experiment is continued for more than a few hours, the water surface may be measurably lowered as a result of evaporation—the water vapour condensing on the inside of the jar. Error from this cause can be eliminated in the following way. A piece of glass tubing is drawn out to a fine capillary, sealed at the end, and inserted through the cork (H, Fig. I). The height of this is adjusted until the lower end is about the same level above the water surface as the tip of the root. Whenever the position of the glass rod which causes contact of the water surface with the root is determined, that which causes contact with the end of H is also noted. Thus, the position of the root tip is measured at successive intervals in relation, not to the water level, but to the end of H, which is a fixed point. Alteration of the water level during the experiment is thus of no importance.

(3) For prolonged experiments it will be found advisable to have a slow stream of air bubbling through the water in the jar. This provides aeration for the root and also ensures that the atmosphere around the root is kept constantly moist.

W. NEILSON JONES.

BOTANICAL LABORATORY,  
BEDFORD COLLEGE,  
*July, 1920.*